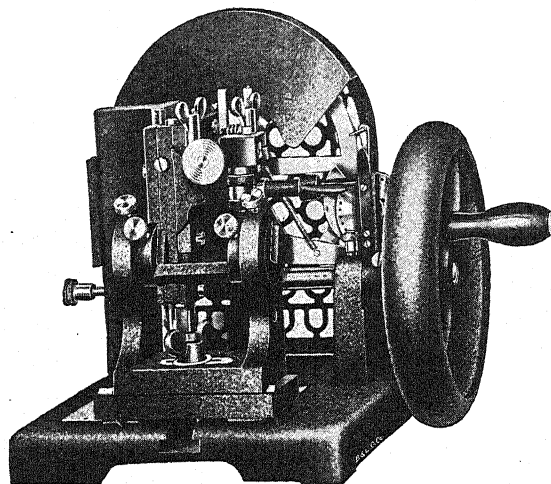


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## VOLUME XI—1924

WITH FORTY-ONE PLATES AND ONE HUNDRED AND FIFTY-NINE TEXT FIGURES

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## ERRATA, VOLUME XI

- Page 28, 9th line from bottom. For *vegans*, read *vagans*.  
Page 154, 12th line from bottom. For *gallegina*, read *galligena*.  
Page 288, line 17. For *Licorice*, read *Lycoris*.  
Page 310. Second line of legend accompanying text figure 8 should read: "metaphase of *C. triflora*; *b*, late heterotypic metaphase of *C. Smithii*; *c*, heterotypic anaphase"  
Page 366, line 8. For *mais*, read *mays*.  
Page 410, line 37; page 411, lines 16, 25, and 30; page 413, line 6; and page 416, line 38.  
For *Phylloxerus*, read *Philoxerus*.  
Page 414, line 19. For *trinerva*, read *trinervia*.  
Page 414, line 25. For *buxifolium*, read *buxifolia*.  
Page 414, line 26. For *Torralbasi*, read *Torralbasi*.  
Page 414, line 34. For *Aschyranthes*, read *Achyranthes*. For (*St.*, *Hill*), read (*St. Hil.*).  
Page 414, line 35. For *maritimus*, read *maritima*.  
Page 415, line 1, and legend accompanying text figure 1, line 1. For *gnaphaloides*, read *gnaphalodes*.  
Page 415, line 1. For *R. Br.*, read (*L.*) *R. & S.*  
Page 415, 8th line from bottom. For *Schultz*, read *Schulz*.  
Page 415, 7th line from bottom. For *Tourneria*, read *Turnera*.  
Page 415, 6th line from bottom. For *Gris.*, read *L.*  
Page 415, 2d line from bottom. For *gnaphaloides*, read *gnaphalodes*.  
Page 416, line 10. For (*L.*) *Pohl.*, read *L.* For (*Bois.*) *Mills.*, read *Boiss.*  
Page 416, lines 10 and 25. For *Omphalia*, read *Omphalea*.  
Page 416, line 11. For *Plumiera*, read *Plumeria*.  
Page 416, lines 12 and 27. For *Fagaria fagaria*, read *Fagara fagara*.  
Page 416, line 13. For *Comoclada*, read *Comocladia*.  
Page 416, line 14. For *subdensata*, read *subdentata*. For *Echitis*, read *Echites*. For *neuriandra*, read *neriandra*.





# AMERICAN JOURNAL OF BOTANY

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No. 1

## HYDROGEN-ION CONCENTRATION GRADIENT IN PLANTS

FELIX G. GUSTAFSON

(Received for publication January 24, 1923)

In connection with some work on the hydrogen-ion concentration and immunity of certain bean varieties to bacterial blight, the writer noticed that there was a difference in the  $H^+$  concentration of the younger and older portions of the plants. This difference in itself was very interesting, and when viewed from the standpoint of the metabolism of the plant it might be considered of considerable importance.

As far as the writer is aware there has been only one reference made to a similar fact. Haas<sup>1</sup> states that the upper five inches of a blue-grass plant in the pollinating stage showed pH 6.11, while the lower 15 inches showed pH 5.92. The upper part of the stem of a sweet clover plant was found to be at pH 6.68, and that of the lower at pH 6.46. The data given by Haas are very much like those found by the writer in the bean plants.

Bush beans were unsatisfactory for any detailed investigation of this matter, and field corn was consequently used for further studies. This is an ideal plant for experiments of this type, because of its long single axis with leaves quite far apart and in a definite series, making it easy to determine the comparative age of the leaves.

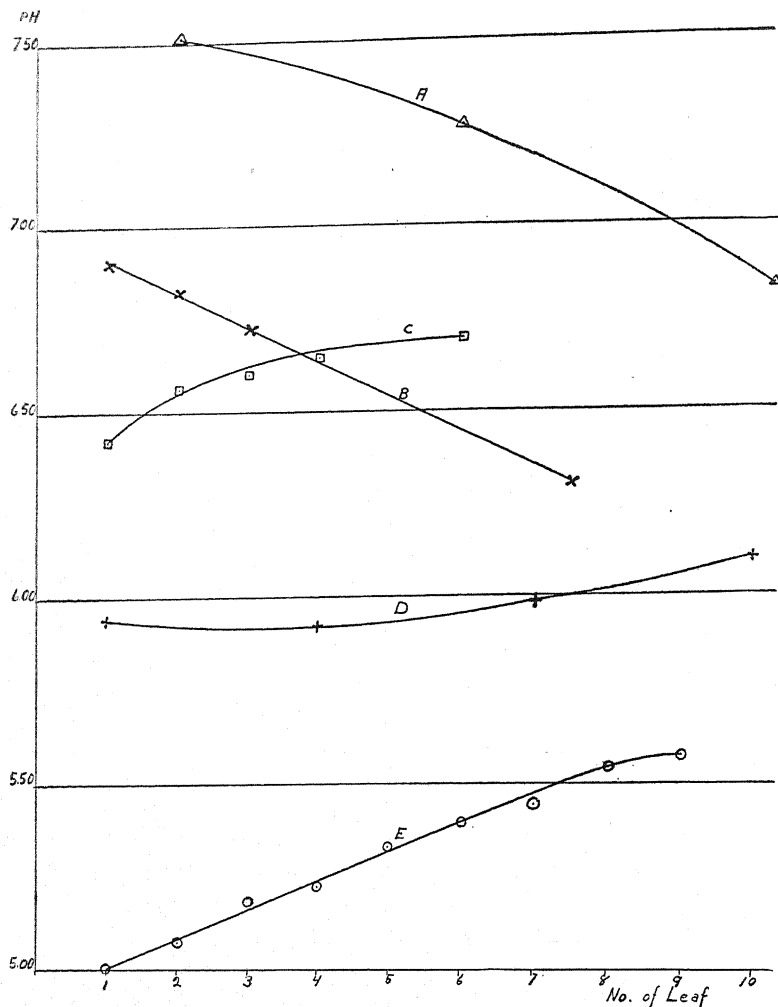
For the first experiments the plants were grown out of doors. When used the plants were a month and a half to two months old. At that time the plants were approximately five feet tall, having on an average 10 leaves. Most of them were in tassel and beginning to silk, though some older plants with nearly mature seeds were also used.

The first experiments were carried out with leaves. Usually about 12 plants were chosen as nearly alike as possible. The determinations began with the oldest leaves and progressed to the youngest, though the reverse order was also tried without any difference in the results. The leaf from the first node was taken from each plant. These oldest leaves were ground coarsely in a food-chopper and the juice was expressed by a hand press, using approximately the same pressure each time. The  $H^+$  concentration

<sup>1</sup> Haas, A. R. C. Studies on the reaction of plant juices. *Soil Sci.* 9: 341-369. 1920.

[The *Journal* for December (10: 515-576) was issued December 31, 1923.]

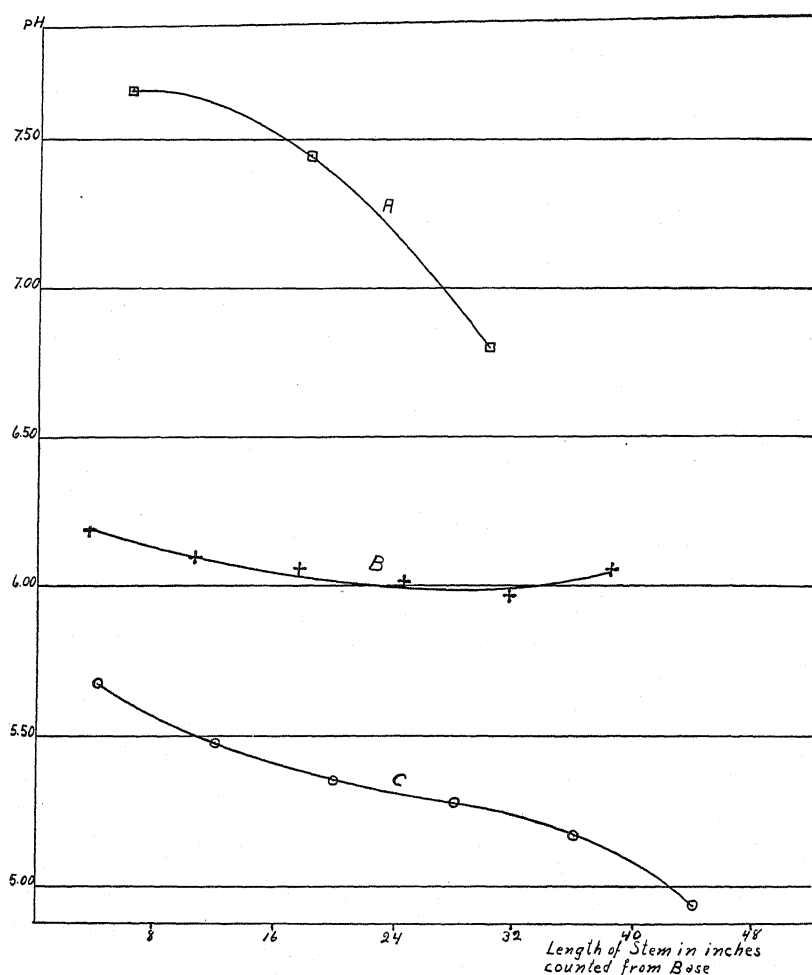
was determined at once as described below. Another set of leaves was being prepared in the same way, while the  $H^+$  determination was being made on the preceding lot.



TEXT FIG. 1. Curves showing the  $H^+$  concentration gradient in leaves. A represents pumpkin, B sunflower, C squash, D pole bean, and E corn.

The  $H^+$  concentration was determined by means of a potentiometer, with an open-type electrode. A closed Clark electrode was used in preliminary experiments, but as no difference in results was noticed it was abandoned on account of its greater complexity. About 10 cc. of juice was used for each determination. The readings were taken 5 minutes after the

electrode was placed in the solution; during this time hydrogen was continually bubbling over the electrode. It had been previously found that this was sufficient time for the electrode to come into equilibrium with the juice. Before and after an experiment the electrode was checked up against a standard buffer solution to see that it was in proper working order.



TEXT FIG. 2. Curves showing the  $H^+$  concentration gradient in stems. A represents pumpkin, B sunflower, and C corn.

From the work on the leaves of corn it was found that, as one proceeds from the lowermost leaves to the uppermost, there is a decrease in  $H^+$  concentration, *i.e.*, an increase in the pH value. It was found that the pH increased with every leaf from base to tip, though not always by the same increment. The lowermost limit was about pH 5.00 while the upper limit was about pH 5.60.

The stems were cut into 20-cm. pieces, usually 6 in all. Four or five stems gave sufficient juice to make the determinations. The lowermost portion was determined first and the others were determined in sequence. The stems gave rather unexpected results. The oldest parts of the stems, unlike the oldest leaves which were attached to those portions of the stem, were found to have a higher pH value than the younger stem portions. In other words, the oldest part of the stem and the youngest leaves had about the same  $H^+$  concentration, while the youngest part of the stem and the oldest leaves were approximately alike. It is rather surprising to find this divergence in parts so intimately connected as a leaf and the stem to which it is attached.

These experiments on corn have been repeated a number of times, with plants grown out of doors in the summer and plants grown in the greenhouse in the winter. There has been only one exception to the above-cited results. That was after several days of heavy rain, when the ground in which the plants were growing became very soggy. For two days in succession experiments were run on the stems, and in all experiments there was no general gradient. The leaves behaved in the usual fashion throughout.

When plants mature the gradient still persists, but there is very much less difference between leaves at two adjacent nodes, especially in the older portions of the plant, and the curve flattens out.

To find whether a gradient in the  $H^+$  concentration was present in other plants than corn, experiments have also been made with squash, pumpkin, pole bean, and sunflower plants. The leaves of squash and pole beans behaved essentially like those of corn, *i.e.*, the older leaves had a higher  $H^+$  concentration than the younger leaves. In the case of the squash the juice was very near the neutral point. The highest that was noticed for squash was pH 6.40, as compared with pH 5.00 for corn. The pole bean is intermediate, at about pH 6.00.

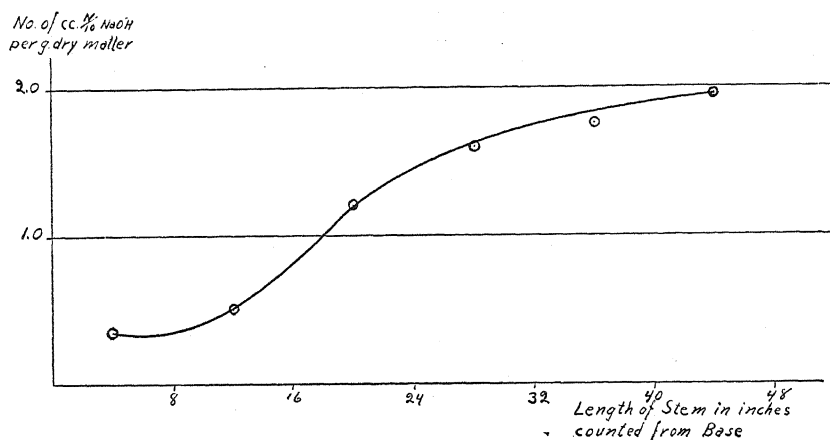
Pumpkin and sunflower leaves gave results the opposite of those above mentioned. In both cases the older leaves had a lower  $H^+$  concentration than the younger. The sunflower leaves ranged from pH 6.90 to pH 6.30. The older leaves of pumpkin are the only leaves so far found that had a juice on the alkaline side of neutrality. In the one experiment that has so far been made the range was from pH 7.50 to pH 6.80.

The stems of the pumpkin and of the sunflower are the only stems that have been tested besides those of corn. In both these the gradient of the stem agrees with their leaves, and also with the corn stem which is the reverse of its leaves.

To what is this difference in  $H^+$  concentration due? It is not probable that it is due to unequal amounts of  $CO_2$  in these parts, because hydrogen was allowed to bubble through the plant juice until there was no further change, indicating that  $CO_2$  equilibrium had been attained. It can not be due to different degrees of dilution of the substances in the cell, as the older

portions always have more dilute juice in them than do the younger and still immature parts, yet these older parts sometimes have a higher  $H^+$  concentration.

The total amount of water-soluble acids has been determined in dried corn stems. The results agree fairly closely with the  $H^+$  concentration of the stems. The lower parts of the stem had very much less total acid than did the upper parts of the stem. The two curves are not identical, and undoubtedly part of the difference is due to some other factor.



TEXT FIG. 3. Curve showing the total water-soluble acidity of dried corn stems at different levels in the stem.

Whether the unequal amount of acid-reacting substances is due to storage, to unequal metabolism, to unequal absorption of cations, or to some other factors is at present impossible to state. When we consider the case of corn it seems unlikely that any one factor would operate alone.

The disappearance of the gradient in the stems and not in the leaves of corn after the heavy rain is probably due to a dilution of the juice with consequent change in the  $H^+$  concentration. As has been shown by Bauer and Haas,<sup>2</sup> and also by Hurd,<sup>3</sup> the stem has a very much lower buffer concentration than the leaves. The excessive amount of water in the soil, the humid conditions following a rain in warm weather, and the cloudy conditions caused the stems to contain more water than usual with the consequent dilution of the weak buffer solution and the upsetting of the  $H^+$  gradient.

<sup>2</sup> Bauer, F. C., and Haas, A. R. C. The effect of lime, leaching, form of phosphate and nitrogen salt on plant and soil acidity, and the relation of these to the feeding power of the plant. *Soil Sci.* 13 : 461-477. 1922.

<sup>3</sup> From paper given at the Boston meeting of the A. A. A. S. Cited with Miss Hurd's permission.

## SUMMARY

The data submitted show that there is a  $H^+$  concentration gradient in corn, squash, pole bean, pumpkin, and sunflower plants.

This gradient is not always in the same direction in different species, or in the leaves and stems of the same species (*e.g.*, corn). In corn, squash, and pole bean the older leaves had a higher  $H^+$  concentration than the younger leaves, while in pumpkin and sunflower the reverse was the case.

The bases of the stems of corn, sunflower, and pumpkin had a lower  $H^+$  concentration than the tops.

DEPARTMENT OF BOTANY,  
UNIVERSITY OF MICHIGAN

## A PRIMITIVE SPOROPHYTE IN MAIZE<sup>1</sup>

WILLIAM H. EYSTER

(Received for publication February 23, 1923)

The production of seeds is a fundamental characteristic of the spermatophytes. In many angiosperms that have been studied, one of the male gametes fuses with the primary endosperm nucleus to form the endosperm nucleus; the other male gamete fuses with the egg to form the oöspore. The endosperm nucleus divides rapidly and the cell containing it develops into the endosperm, while the oöspore develops more slowly into the proembryo and sporophyte. Sooner or later, but while it is still contained within the integuments, the embryo stops its growth and in many species enters a state of dormancy. In this condition, which is called the seed stage, the sporophyte is well adapted to pass through periods that are unfavorable for growth. In some plants the sporophyte becomes inactive while there is still much of the endosperm unused, while in others development continues until the endosperm is entirely absorbed by the sporophyte and further growth seems to be prevented by the seed coat.

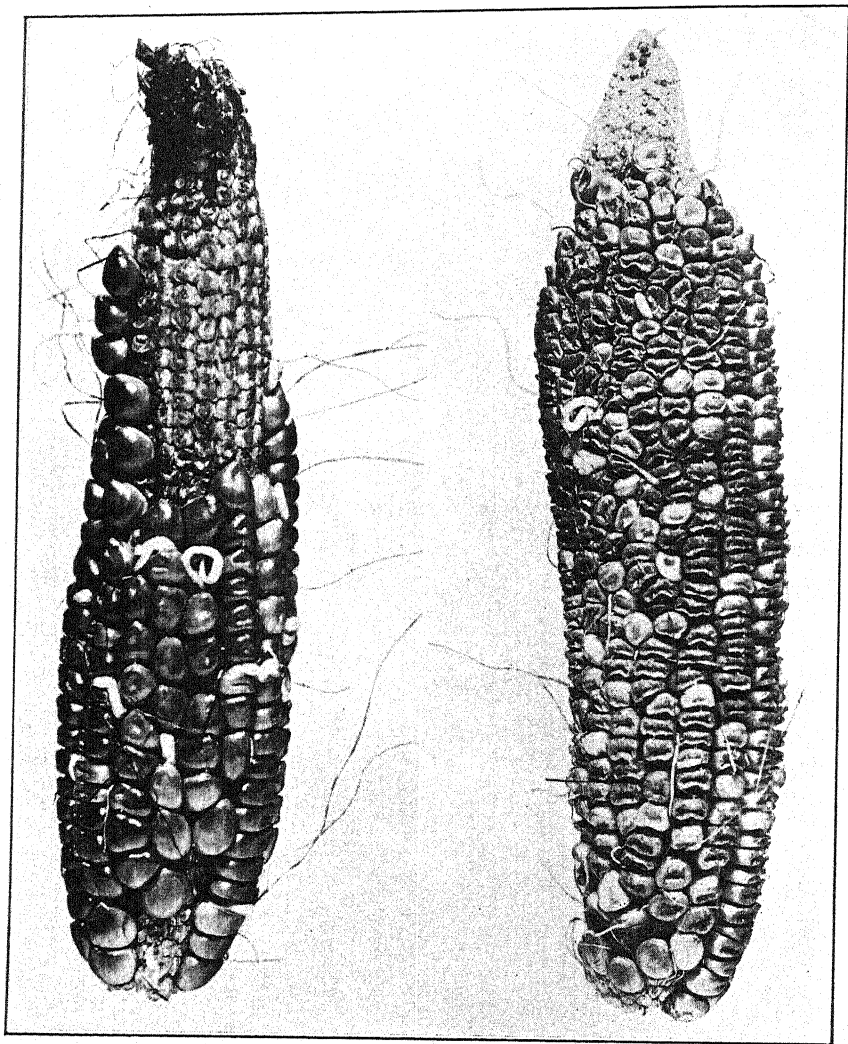
The delay in the germination of mature seeds, Crocker (1916) finds, is due either to (1) a seed coat that is impervious to water and gases, or hard and too resistant to the developing embryo, or to (2) the inherent nature of the sporophyte itself. There are many seeds in which both categories of causes are operative. Is stoppage of growth of the embryo in the seed stage initiated by the same factors that cause delay in germination when mature seeds are placed under conditions favorable for growth? In the Leguminosae it is usually the hard and impervious seed coat which causes the delay in the germination of mature seeds. In these seeds the growth of the embryo continues until the endosperm is exhausted and the seed coat is completely filled, and while the loss of water is doubtless the principal factor, it may well be that the seed coat plays some part in the stoppage of growth of the sporophyte in the formation of seeds. In this case it should be possible to provide conditions that would cause the embryo to continue its development to a mature plant without undergoing a period of inactivity in the seed stage.

In many plants it has been found by Goebel (1898) that at the time of ripening the seeds contain immature sporophytes ranging all the way from a group of undifferentiated cells to a mature embryo. The apparent dormancy in these seeds is in reality a period of slow development. If such seeds are kept under favorable growth conditions, the embryos develop directly into new plants. There are other plants in which the sporophyte

<sup>1</sup> The investigation reported in this paper was made in connection with genetic studies of maize in the Department of Field Crops, Missouri Agricultural Experiment Station.



develops to be a mature embryo and then for some reason stops growing and becomes dormant. It is possible that suitable conditions provided at the proper time would prevent this type of dormancy; but, once dormant,



TEXT FIG. 1. *a* (left), a self-pollinated ear segregating primitive sporophytes. *b* (right), a self-pollinated ear segregating primitive sporophytes and a pale yellow endosperm. Note that these two characters are closely correlated.

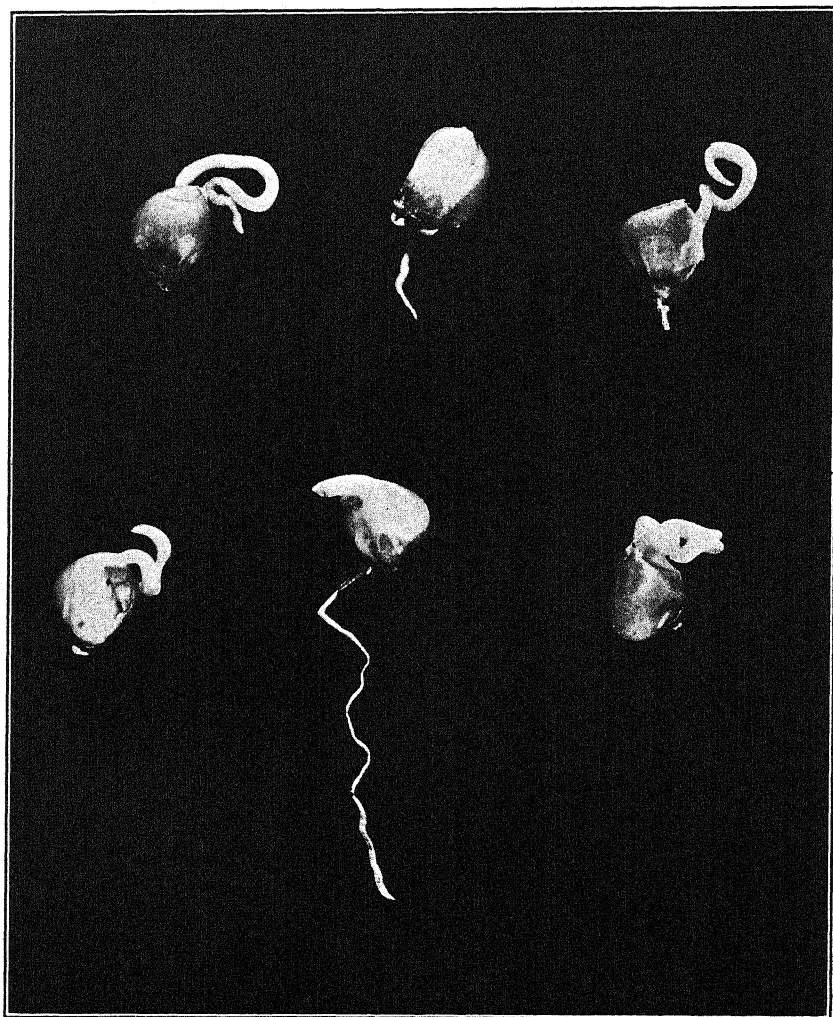
a sporophyte fails to develop; even under optimum conditions, until it passes through an after-ripening period during which time some fundamental chemical changes occur, as found by Eckerson (1913) in embryos of *Craetagus mollis*.



In maize the growth of the embryo usually stops while there is still much of the endosperm unused. This stoppage of growth seems to be caused largely by an insufficient water supply, for if developing ears are kept under suitable moisture conditions, as in the case of plants that are broken down so that the ears lie on wet soil or are partially imbedded therein, the sporophytes appear to have a continuous growth from the fertilized egg to the new plant.

#### DESCRIPTION OF A PRIMITIVE SPOROPHYTE IN MAIZE

Whatever may be the real cause of the stoppage of growth in the seed stage of the maize sporophyte, I have in my cultures a type which fails to



TEXT FIG. 2. Kernels with primitive sporophytes, *i.e.*, sporophytes that have a continuous development from the fertilized egg to the new plant.

stop growing in the seed stage under natural conditions. It has a continuous development, on normal erect plants, from the fertilized egg to the new plant, and in this respect is like the sporophytes of the bryophytes and pteridophytes and of all primitive plants that have a multicellular sporophyte. Because of this resemblance I have designated it *primitive sporophyte*. If ears which bear primitive sporophytes are allowed to ripen on the plant, the young plants die in various stages of development, in consequence



TEXT FIG. 3. Two albino plants which were grown on wet filter paper in petri dishes from kernels in which the sporophytes failed to become dormant in the seed stage.

of the lack of water. In figure 1 are shown ears with primitive sporophytes which were harvested while the husks were still green and there was sufficient moisture in the ears to enable the plants to live. In many kernels the plants make a considerable growth so that they become much coiled and doubled up before the pericarp is ruptured, as is shown on the ears in figure 1a, and by the kernels illustrated in figure 2. Sprouted kernels were removed from a number of such ears and placed on wet filter paper in petri dishes where the plants made a rapid growth. Some five hundred kernels

were thus tested, and every plant proved to be pure albino and died when the food reserve in the endosperm was exhausted. Two of these albino plants are shown in figure 3. The primary root develops first, as is shown in the middle vertical row in figure 2, and soon dies from lack of moisture, but the plants grown in petri dishes soon put out, from the stem immediately above the kernel, secondary roots for the absorption of water.

#### INHERITANCE OF PRIMITIVE SPOROPHYTE

Three ears of maize grown in the summer of 1921 were found to have kernels scattered over their surfaces that had sprouted prematurely and died. It was found that the number of normal and of sprouted kernels approached very closely the expectancy for a 3:1 ratio, which fact indicated that the sprouted kernels were due to a recessive Mendelian factor. Unfortunately the actual data from these three ears are not now available. The viable seeds from each ear were planted in the field and the plants were self-pollinated, with the results given in table 1. There were 1005 plants

TABLE 1. *F<sub>3</sub> Progenies of Crosses Equivalent to Normal × Primitive*

Pedigree Numbers	Ears with Normal Kernels only	Ears with Normal and Sprouted Kernels	Total	Ratio
3236	105	231	336	0.93:2.07
3237	140	259	399	1.05:1.95
3239	87	183	270	0.97:2.03
Total.....	332	673	1005	0.99:2.01

which produced ears, of which 332 bore only normal kernels and 673 had both normal and sprouted kernels. These are *F<sub>3</sub>* progenies of crosses equivalent to *normal × primitive*. In each case the numbers observed are in close agreement with those expected. A summary comparison follows:

	Ears with Normal Kernels	Ears with Normal and Sprouted Kernels	Total	Ratio
Observed.....	332	673	1005	0.99:2.01
Calculated (1:2).....	335	670	1005	1.00:2.00
Deviation.....	-3	+3		

In all there were 673 ears with both normal and sprouted kernels. Because of the extremely unfavorable field conditions under which the plants were grown, most of the ears had only a small number of kernels. Thirteen of the largest segregating ears were harvested before they were entirely ripened, and the numbers of normal and sprouted kernels were counted. The results are given in table 2. The relation of normal to sprouted kernels

TABLE 2.  $F_2$  Progenies of Crosses Equivalent to Normal  $\times$  Primitive

Pedigree Numbers	Normal	Primitive	Total	Ratio
3236-4	228	94	322	2.83 : 1.17
5	184	55	239	3.08 : 0.92
12	303	99	402	3.01 : 0.99
18	220	79	299	2.93 : 1.07
19	173	51	224	3.10 : 0.90
21	170	52	222	3.06 : 0.94
22	354	114	468	3.03 : 0.97
23	56	47	103	2.18 : 1.82
3237-4	210	64	274	3.07 : 0.93
12	232	90	322	2.88 : 1.12
16	382	135	517	2.95 : 1.05
3239-1	415	137	552	3.00 : 1.00
2	157	51	208	3.00 : 1.00
Total.....	3084	1068	4152	2.97 : 1.03

of the individual ears ranges from 2.83 : 1.17 to 3.10 : 0.90, with an average ratio of 2.97 : 1.03. There were 3084 normal kernels and 1068 sprouted kernels, making a total of 4152. A comparison between the observed and expected numbers is here given:

	Normal	Primitive	Total	Ratio
Observed.....	3084	1068	4152	2.97:1.03
Calculated (3 : 1).....	3114	1038	4152	3.00:1.00
Deviation.....	-30	+30		

These data show that this unusual maize sporophyte which does not stop its development in the seed stage is inherited as a recessive Mendelian character. The factorial symbol,  $Pm\ pm$ , is used to designate *primitive sporophyte*.

#### RELATION OF PRIMITIVE SPOROPHYTE TO A PALE YELLOW ENDOSPERM AND A WHITE SEEDLING

Eleven of the 13 ears studied that segregated primitive sporophytes also segregated a pale yellow endosperm. There is a close correlation between this pale yellow endosperm and the primitive sporophyte. This can be seen on the ear shown in figure 1b. The kernels from each ear were classified, with the results recorded in table 3. No tests have yet been made to determine whether the pale yellow endosperm here concerned is identical with the pale yellow endosperm of Emerson.

TABLE 3. *F<sub>2</sub> Progenies of Crosses Equivalent to Yellow × Pale Yellow Primitive*

Pedigree Numbers	Yellow Endosperm		Pale Yellow Endosperm	
	Normal Sporophyte	Primitive Sporophyte	Normal Sporophyte	Primitive Sporophyte
3236-4	226	1	2	93
5	184	0	0	55
12	300	6	3	93
18	216	2	4	77
19	169	0	4	51
21	170	0	0	52
23	55	1	1	46
3237-4	205	4	5	60
12	231	3	1	87
3239-1	413	1	2	136
2	157	0	0	51
Total . . . . .	2326	18	22	801

These results may be explained by assuming a close linkage between the factors for primitive sporophyte and for pale yellow endosperm. A comparison between the observed numbers and the numbers expected with 1.26 percent crossing over follows:

	Yellow Endosperm		Pale Yellow Endosperm	
	Normal	Primitive	Normal	Primitive
Observed . . . . .	2326	18	22	801
Expected with 1.26% crossing over . . . . .	2356	20	20	772
Deviation . . . . .	-30	-2	+2	+29

All the primitive sporophytes tested in the petri dishes were pure albinos. The 22 normal kernels with pale yellow endosperm were also tested, and 21 of them produced green plants, but one an albino plant. These kernels were tested several months after harvesting the ears so that no mistake was made in their classification. This observation indicates that primitive sporophyte and albino seedlings are due to separate factors which are closely but not completely linked. Tests have not been made to determine whether the albino seedling is the same as the one reported by Lindstrom (1918).

#### SUMMARY

In the seed plants growth of the sporophyte is interrupted and in many species undergoes a period of dormancy in the seed stage. In this condition it is well adapted to pass through an unfavorable growth period. In maize a sporophyte has been found which has a continuous development from the

fertilized egg to the new plant. Under natural conditions this proves lethal to the plant because of an insufficient water supply to maintain life. This continuous development of the sporophyte, which is characteristic of the sporophytes of the more primitive plants, is inherited in maize as a simple Mendelian recessive. The factor for primitive sporophyte appears to be closely linked with factors for pale yellow endosperm and albino seedling.

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## SOME EFFECTS OF CERTAIN CALCIUM SALTS UPON THE GROWTH AND ABSORPTION OF CITRUS SEEDLINGS<sup>1</sup>

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The ash of citrus trees is relatively rich in calcium, and the trees are especially sensitive to the amount of this ion absorbed. Kelley and Cummins (2) have shown that with age citrus leaves grow richer in calcium. The writers (3 and 4) have shown some of the specific effects of calcium in the presence as well as in the absence of large amounts of sodium salts.

A few years ago Breazeale (1) showed the accelerating influence of calcium carbonate upon the root growth of citrus seedlings in distilled water as well as in the presence of deleterious factors. The ameliorating action of calcium carbonate in the presence of toxic substances was somewhat similar to that observed by Schreiner and Reed (5) in the case of wheat seedlings. In the present paper some data are presented to show the way in which certain calcium salts affect growth of citrus seedlings. The analyses of seedlings grown in solutions of calcium salts may show something of the effect of anions upon absorption by these plants.

The experiments were conducted with citrus seedlings in water cultures. A single culture consisted of three plants in a quart glass jar. From 36 to 54 plants were employed in each experiment. The distilled water used in preparing all solutions was freed from traces of copper and other toxic materials by shaking with washed carbon-black and subsequent filtering.

The first experiment was designed to compare the effects of calcium chlorid and calcium nitrate singly upon growth and absorption. African sour orange (*Citrus aurantium* L.) seedlings were used. The solutions in each case contained 159 p.p.m. Ca. The cultures were grown for 42 days without renewing the solutions; distilled water was, however, added from time to time. During this period the materials in the cotyledons seemed sufficient to meet the needs of the young plants.

The roots in the calcium chlorid solutions grew slowly, and the lateral rootlets were less than half a centimeter in length. In the calcium nitrate solution the roots made vigorous growth and the lateral rootlets were from 5 to 10 cm. in length. The green and dry weights of the plants (table I) show the superiority of those grown in the calcium nitrate solution.

In both solutions there was an increase in the acidity (decrease in pH) during the period of the experiment.

<sup>1</sup> Paper no. 108, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

TABLE I. *Growth and Composition of Orange Seedlings Grown in Solutions of  $\text{CaCl}_2$  and  $\text{Ca}(\text{NO}_3)_2$* 

Seedlings	No. of Seedlings Used for Analysis	Weight in Grams (based on 100 seedlings)					Reaction Values Computed from Ash Analyses	
		Green	Dry (Dried at 60°-70° C.)	Ash	Ca	Cl	Ca	Cl
At the start.....	54	39.63	6.5166	0.3634	0.0228	0.0039	.00110	.00011
After growing 42 days in $\text{CaCl}_2$ solution....	51	35.30	8.8186	0.5659	0.0928	0.0439	.0046	.00121
After growing 42 days in $\text{Ca}(\text{NO}_3)_2$ solution	52	57.69	13.0016	0.9446	0.2085		.0104	

Table I gives the determinations which were made. One lot of 54 seedlings was weighed and analyzed at the time the cultures were started, and is used as a basis for comparison. The reaction values were computed from the difference between the composition of the seedlings at the start and that at the termination of the experiment. The green weight of seedlings grown in calcium chlorid solution was 0.9 of their initial weight; in calcium nitrate solution it was 1.5 times their initial weight. These relations are not surprising when we consider the usual effect of nitrates upon the growth of seedlings. The dry weight produced in the calcium chlorid solution was 1.4 times the original dry weight, and in the calcium nitrate solution it was 2.0 times the original dry weight.

It is evident from table I that different salts of calcium are not equally efficient in stimulating the growth of citrus seedlings in water cultures containing carbon-treated distilled water plus a single calcium salt. The experiment shows that in cultures in which single salts are employed the nature of the anion is of very considerable importance.

The amounts of calcium in the ash of the different lots of seedlings showed that an active absorption of that ion had taken place. In each lot the weight of Ca was several times as great as in the original seedlings. There was a percentage gain in Ca as well as an absolute gain, as shown by the fact that the Ca content of the ash of the original seedlings was 6 per cent, while in seedlings grown in calcium chlorid the calcium content was 16 per cent of the ash and in calcium nitrate it was 22 percent.

The reaction values of Ca and Cl in the original seedlings showed that the ratio of the two ions was about 10 to 1. The values of the net amount of Ca and Cl absorbed by seedlings grown in calcium chlorid solution were in the ratio of 3 to 1. The figures in table I show that the seedlings absorbed Ca in larger amounts than Cl from the calcium chlorid.

The growth of plants in solutions of calcium salts of equivalent calcium concentration is so evidently dependent upon the nature of the anion that



it is necessary to supplement the above-described experiment with another series in which all necessary anions were present.

In the next experiment the effect of four calcium salts upon root growth of rough-lemon (*Citrus limonia* Osbeck) was observed in solutions containing all the remaining essential ions. The object of this experiment was to learn whether the favorable effects of calcium carbonate in distilled water observed by Breazeale (1) could be obtained with other Ca salts in the presence of a supply of other necessary ions. A calcium-free solution was placed in culture jars to which the various calcium salts, in amounts shown in table 2, were added. The carbonate and sulphate were added as dry salts, the others were added as solutions. Some of the calcium carbonate was always present in the solid phase, but all the calcium sulphate eventually went into solution.

The rough-lemon seedlings were allowed to grow eleven days in the cultures. No development of lateral rootlets occurred during this period. Table 2 shows the average increment in length of roots for each culture jar during the eleven days.

TABLE 2. *Increments in Length of Roots of Rough-lemon Seedlings during an 11-day Period in Nutrient Solutions Containing Various Calcium Salts*

Complete Nutrient Solution with K Replacing Ca, plus Total Ca as follows:

Culture Jar	159 p.p.m. Ca as $\text{Ca}(\text{NO}_3)_2$	159 p.p.m. Ca as $\text{CaSO}_4$	159 p.p.m. Ca as $\text{CaCl}_2$	159 p.p.m. Ca as $\text{CaCO}_3$	5 p.p.m. Ca as $\text{CaCl}_2$
	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>	<i>Cm.</i>
1	2.37	2.47	3.13	2.0	2.07
2	3.07	2.57	2.67	2.23	1.87
3	2.27	3.40	2.53	2.60	1.47
4	2.57	2.10	2.13	2.03	1.40
5	2.73	2.07	3.10	2.63	2.10
6	3.13	2.47	3.07	1.83	1.70
7	3.80	2.50	2.07	1.37	2.00
8	2.93	3.50	2.67	2.03	2.27
9	2.13	2.90	2.13	3.47	2.03
10	2.0	3.00	3.07	2.57	2.63
11	1.70	2.87	1.93	2.87	2.33
12	2.50	1.70	2.90	1.97	2.33
Average increment per seedling	$2.64 \pm .10$	$2.63 \pm .10$	$2.62 \pm .08$	$2.30 \pm .10$	$2.02 \pm .07$

The average of each series is given at the bottom of the table. The values for the nitrate, sulphate, and chlorid are practically identical. The average for the roots in the carbonate series is somewhat below the averages of the first three salts, but if we disregard differences of less than three times the probable error, the difference from the other series is not very significant. We seem justified therefore in the assumption that other salts of calcium are equal, if not superior, to the carbonate in producing root growth of citrus seedlings in the presence of the other essential ions.

The culture solution employed had a pH of 5.2. It is practically certain that the addition of calcium carbonate reduced the acidity to some extent, although no determinations of the pH were made. There is no evidence from our results that decreased acidity had any tendency to increase growth of citrus roots.

The last series of cultures shown in table 2 received 5 p.p.m. Ca as calcium chlorid for comparison with the series which received calcium carbonate. It was thought that this concentration would be comparable with the amount of Ca in solution at any given time in the calcium carbonate. We must remember, however, that, so long as the solid phase was present, the carbonate solution would be saturated for the temperature and pressure which prevailed. The concentration, though low, would therefore be maintained in spite of the absorption of Ca ions by the plants. The concentration of Ca ions in the calcium chlorid series would, on the contrary, decrease to a minimum. There is no doubt that the plants in the low concentration of calcium were restricted in their growth by lack of calcium.

#### SUMMARY

Our results indicate that citrus seedlings grown in a calcium chlorid solution will absorb more calcium cations than chlorid anions.

In the concentrations employed, the growth of roots was influenced more by the amount of calcium in solution than by the character of the anion with which the calcium cation was combined in the salt used.

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# A STUDY OF FACTORS PROMOTING PYCNIDIUM-FORMATION IN SOME SPHAEROPSIDALES

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## INTRODUCTION

A fungus, like any living organism, is a composite of hidden potentialities which may not manifest themselves unless subjected to the proper environmental stimuli. The same fungus under the influence of one group of environmental factors may show the characteristics of one specific organism, but when subjected to the action of another group of factors it may reveal different morphological peculiarities. It is highly essential, therefore, to investigate the physiological relations of fungi in order to classify them properly. The Sphaeropsidales, which contain a large number of interesting as well as economically important forms, have received but scanty attention from physiological investigators. Although the literature on the pathogenic relationship of these fungi is quite extensive, yet, since the papers emphasize the fungi as causal organisms of disease rather than as complex living things possessing many other characteristics, such accounts are necessarily specific in nature. It is the object of this paper, therefore, to study under controlled laboratory conditions some twenty representative members of the Sphaeropsidales and to organize in groups the typical reactions of these forms particularly from the standpoint of pycnidium-production.

This work has been done in the cryptogamic laboratory of the University of Michigan under the direction of Dr. C. H. Kauffman, for whose inspiring guidance I wish to express my deep gratitude.

## ORGANISMS

The following is a list of the organisms which were used in the experiments outlined in this paper. Unless otherwise stated, all of them have been collected, identified, and placed in pure culture by the writer.

1. *Phyllosticta opuntiae* Sacc. & Speg. On *Opuntia* sp. State College, N. M., January, 1920.
2. *Phoma urens* E. & E. On *Populus* sp. Chelsea, Mich., May, 1921.
3. *Sphaeronema pruinosum* B. & C. On *Rhus glabra* L. Ann Arbor, Mich., August, 1921.
4. *Sphaeronema spurium* (Fr.) Sacc. On *Prunus americana* Marsh. Ann Arbor, Mich., June, 1921.
5. *Vermicularia circinans* Berk. On the bulbs of *Allium Cepa* L. Ann Arbor markets, October, 1920.

6. *Plenodomus destruens* Harter. On *Ipomoea Batatis* Poir. Subcultures made from material sent to Ann Arbor by Dr. L. L. Harter.
7. *Cytospora mendax* Sacc. & Roum. On branches of *Quercus* sp. Chelsea, Mich., May, 1921.
8. *Naemosphaera* sp. On leaves of *Typha* sp. Francisco, Mich. Collected by Lee Bonar, 1920.
9. *Coniothyrium concentricum* (Desm.) Sacc. On leaves of *Yucca macrocarpa* Engelm. State College, N. M., January, 1920.
10. *Ascochyta nymphaeae* (Passer.) Hedw. On leaves of *Nymphaea advena* Ait. Whitmore Lake, Mich., October, 1920.
11. *Kellermania yuccagena* E. & E. On leaves of *Yucca glauca* Nutt. State College, N. M., January, 1920.
12. *Stagonospora collapsa* (Cke. & Ell.) Sacc. On twigs of *Acer Negundo* L. Ann Arbor, Mich., June, 1921.
13. *Stagonospora gigantea* Heald & Wolf. On leaves of *Yucca elata* Engelm. State College, N. M., January, 1920.
14. *Hendersonia* sp. On leaves of *Rhus glabra* L. Ann Arbor, Mich., June, 1921.
15. *Sphaerographium fraxini* (Pk.) Sacc. On branches of *Fraxinus americana* L. Ann Arbor, Mich., July, 1921.
16. *Endothia parasitica* (Murr.) Anders. On branches of *Castanea dentata* (Marsh) Borkh. Syracuse, N. Y. Collected and identified by Dr. A. H. W. Povah, November, 1920.
17. *Ollula* sp. On leaves of *Rhus glabra* L. Ann Arbor, Mich., October, 1920.
18. *Melanconium betulinum* Schm. & Kz. On branches of *Betula* sp. Terre Haute, Ind., April, 1921.
19. *Pestalozzia guepini* Desm. On leaves of *Hevea brasiliensis* Muell. Kisaram, Sumatra, August, 1918. Collected, identified, and isolated by Dr. Carl D. La Rue.
20. *Steganosporium acerinum* Pk. On branches of *Acer grandidentatum* Nutt. Organ Mountains, N. M., June, 1920. Identified by Mr. Andrew Archer.

#### NUTRIENT MEDIA

It was realized that a purely synthetic nutrient solution would probably fail to induce uniformly good pycnidium-formation in all the foregoing twenty organisms, because it usually seems to lack the mysterious something that is found associated with certain complex organic media. Consequently an effort was made to find a suitable organic preparation that could be combined with some other favorable nutrient solution. Malt extract has been widely used in Europe and found advantageous for the purpose in question. It forms the chief constituent of Blakeslee's agar which has been found to be an ideal medium for the culture of Mucors. Malt extract was selected, therefore, to furnish the unknown factor mentioned above. Since, furthermore, maltose is the dominating type of sugar in the grains which constitute favorable culture media for a large number of fungi, maltose was chosen as the chief carbohydrate source. Peptone was found preferable for the nitrogen supply. With dihydrogen potassium phosphate and magnesium sulphate serving as sources for the essential inorganic salts, there remained only the task of establishing the proper proportions of the various ingredients until a good nutrient solution could be obtained. The following

formula was found to be the best, and this has been used exclusively throughout the experiments outlined in this paper:

Dihydrogen potassium phosphate.....	1.25	grams
Magnesium sulphate.....	0.625	"
Peptone.....	0.625	"
Maltose.....	6.25	"
Malt extract.....	6.25	"
Distilled water.....	1,000	cc.

The solution was heated in a double boiler or in Arnold's sterilizer for fifteen minutes, filtered, and then autoclaved at ten pounds' pressure for fifteen minutes. If a solid medium is desired, 1.5-2 percent agar agar may be added to the solution. The agar agar should be melted in a separate lot of water before it is added to the nutrient portion.

### METHODS

Saprolegnia and other related fungi that normally live in water can best be grown and studied in aqueous solutions or in liquid media, whereas wood- and vegetable-inhabiting organisms like the Sphaeropsidales require solid media for their normal development. Klebs and his followers experienced no trouble in growing algae or water molds in liquid media and in transferring them from one solution to another. But how could the same method be used in the case of fungi growing in test tubes and on solid substrata; and how could these cultures be washed and transferred to another solution? Coons (4) used filter-paper cones as a base for his cultures. He placed these cones in glass capsules which contained a nutrient solution, and the filter paper, remaining moist through capillary action, served as a satisfactory substratum. The same method was at first tried by the writer, but many organisms failed to confine themselves to the cone, and, growing down into the solution, filled the capsules with a mass of dense and moist mycelium. A modification of Coon's method which the writer has described in another paper (17) gave comparatively better results. Filter-paper cups were made and then placed in capsules which contained distilled water. After a thorough sterilization in the autoclave, a nutrient agar was poured into these cups. When the fungi were transferred to this agar, they confined themselves largely to the cup. This method, however, was not found to be practical for the requirements of the present investigations; consequently, a method was developed by which pieces of filter paper were folded into small pads and placed in a nutrient solution where they absorbed and retained enough food to serve the needs of the growing organism, thus eliminating the tendency of the mycelium to grow into the liquid. Subsequent experiments proved this an excellent procedure. In this method, circular glass capsules 5 cm. in diameter and of 25-cc. capacity, and Whatman filter paper no. 1, 12.5 cm., take the place of test tube and agar agar respectively. Each sheet of filter paper is cut into four equal parts, each of which is

moistened with distilled water and then folded to give it a disc-like appearance, two centimeters in diameter and three to four millimeters in thickness. This moist pad is then placed in the palm of one hand and firmly pressed with the thumb of the other hand, thus giving it a concave-convex form. Then it is dried either in the open air or in a drying oven, placed in the capsules with the concave side down, and sterilized in the autoclave. A few cubic centimeters of any desired solution may then be added. Each pad absorbs about one half cc. of solution, while a part of the remaining liquid is attracted to and retained in the hollow of the pad. This pocket-like space draws upon the remainder of the solution in the capsule as the growing organism gradually uses up the ingredients. The fungi make a good growth on these filter-paper pads, eventually covering them with numerous fruiting bodies. The entire culture with its filter-paper pad can be removed at any time from the capsule by means of a flamed forceps, transferred to a series of larger capsules containing distilled sterile water, washed thoroughly, and transferred again to another solution or to distilled water in another capsule. All these operations can be performed without injuring the mycelium. This is an important point that should not be overlooked, because, if mycelium is injured by cutting or otherwise, other factors will enter into the experiments and complicate matters.

The filter-paper-pad culture has another advantage: after the fungus has completed its growth and reproduction, the pads can be taken out of the capsules, dried in the open air, and pasted on cardboards, thus forming a herbarium of pure cultures which, in most cases, retain their natural condition and individual characteristics perhaps for a long time.

*Isolations.* The dilution method of single-spore cultures has been followed during the isolation of all the organisms listed in this paper.

*Symbols.* All data given in the tables of this paper are expressed by numerals; the Roman numerals of each table represent, each, one type of experimentation, while the Arabic numerals indicate the quantity of fruit bodies formed. The number of pycnidia is expressed on the percentage basis because it affords a wider margin for making estimates. The Arabic numeral 5 is taken as the smallest unit, and 100 as the largest. The numeral 5, therefore, means very poor, and, according to the nature of the fungus, it may represent only one, or as many as forty or fifty fruit bodies. Similarly, 100 means very good, and may represent less than fifty or many more than one hundred pycnidia in each culture. No attempt was made to count the actual number of fruit bodies, for, after all, it is the relative quantity of pycnidia that is important. After one has made thousands of cultures and has become accustomed to the behavior of the individual organisms under their best and their poorest conditions for fruiting, one does not find it very difficult to make comparative estimates with a good deal of accuracy.



## THE EXPERIMENTS

The experimental work is divided into two phases: first, the preliminary work, in which such factors as light, temperature, oxygen supply, solidity of the substratum, transpiration, adsorption, and the reaction of the medium have been tested for the purpose of clearing the way to the more definite portion of the problem; second, the principal work, which deals for the most part with food-concentration and its relation to pycnidium-formation in these fungi.

## Light

Two sets of cultures were made; one was kept in diffused light, and the other was placed in a large dark chamber five feet wide, six feet long, and six feet high. Holes in the sides and on the top provided ample circulation of air, although they were loosely plugged with cotton so as to prevent the entrance of light. This chamber was in the same room where the experiments with the diffused light were conducted, and had, therefore, approximately the same temperature. Thus, all factors except light being alike, the results shown in the first two columns of table I are to be attributed to the presence or the absence of light.

I. Cultures were kept in diffused light and at room temperature.

II. Cultures were kept in the dark and at room temperature.

III. Cultures were kept at ordinary room temperature for twenty-four hours; then they were transferred to the dark at a constant temperature of 30° C.

IV. Cultures were kept at ordinary room temperature for twenty-four hours; then they were transferred to the dark at a constant temperature of 8° C.

The keeping of the cultures at ordinary room temperature for a given length of time before transferring them to a high or to a low temperature

TABLE I. *The Effect of Light and Temperature*

Organisms	I. Light	II. Dark	III. 30° C.	IV. 8° C.
<i>Ascochyta nymphaeae</i> . . . . .	60	30	65	45
<i>Coniothyrium concentricum</i> . . . . .	25	10	15	10
<i>Cytospora mendax</i> . . . . .	25	20	35	5
<i>Endothia parasitica</i> . . . . .	15	15	30	5
<i>Hendersonia</i> sp. . . . .	20	0	0	0
<i>Kellermania yuccage</i> a . . . . .	15	10	20	5
<i>Melanconium betulinum</i> . . . . .	20	20	0	0
<i>Naemospora aera</i> sp. . . . .	25	25	40	0
<i>Ollula</i> sp. . . . .	10	10	10	5
<i>Pestalozzia guepini</i> . . . . .	20	10	20	0
<i>Phoma urens</i> . . . . .	10	5	15	0
<i>Phyllosticta opuntiae</i> . . . . .	40	5	40	0
<i>Plenodomus destruens</i> . . . . .	30	30	35	5
<i>Sphaerographium fraxini</i> . . . . .	10	0	5	0
<i>Sphaeronema spurium</i> . . . . .	20	5	20	5
<i>Sphaeronema pruinosum</i> . . . . .	25	5	0	0
<i>Stagonospora collapsa</i> . . . . .	20	10	20	10
<i>Stagonospora gigantea</i> . . . . .	10	10	10	5
<i>Steganosporium acerinum</i> . . . . .	15	10	15	5
<i>Vermicularia circinans</i> . . . . .	40	30	30	20

was for the purpose of eliminating any possible hindering influence exerted by the other temperatures upon the germination of the spores.

Only two organisms, *Hendersonia* sp. and *Sphaerographium fraxini*, failed to form pycnidia in the dark, and while *Plenodomus destruens*, *Naemosphaera* sp., *Stagonospora gigantea*, *Endothia parasitica*, *Melanconium betulinum*, and *Ollula* sp. remained indifferent, the amount of fruiting of the rest of the organisms was reduced.

### Temperature

The chief reason for working with the temperature factor at all was to supplement the experiments with light, and no attempt was made, therefore, to determine the temperature limits of the organisms. An examination of column III of table 1 shows that in some cases a higher temperature not only replaces the effect of light, but can become a more efficient agent for the promotion of fruiting. *Sphaerographium fraxini*, which failed to fruit in the dark and at room temperature, was able to form a few pycnidia at 30°C., even in the absence of light. *Ascochyta nymphaeae*, *Cytospora mendax*, *Endothia parasitica*, *Kellermania yuccagena*, *Naemosphaera* sp., *Plenodomus destruens*, and *Phoma urens* formed more pycnidia at a higher temperature in the dark than they did in the light and at room temperature. *Coniothyrium concentricum*, *Sphaerographium fraxini*, and *Vermicularia circinans* formed fewer pycnidia at 30°C. than they did in the light; *Hendersonia* sp., *Melanconium betulinum*, and *Sphaeronema pruinosum* failed to fruit, while the remaining organisms were indifferent.

*Hendersonia* sp., *Melanconium betulinum*, *Naemosphaera* sp., *Pestalozzia guepini*, *Phoma urens*, *Phyllosticta opuntiae*, *Sphaerographium fraxini*, and *Sphaeronema pruinosum* failed to fruit at 8°C. in the dark. When, however, these eight organisms were transferred into the light but still kept at 8°C., all of them formed pycnidia after a time.

### Oxygen Supply

When one is working with hundreds of cultures at a time, there is always a natural tendency to crowd the cultures in such a way as to interfere with a free air supply. In order to ascertain, therefore, the influence of a lack of free exchange of air and a consequent accumulation of carbon dioxide upon the fruiting of the organisms, the following experiment was arranged: two series of cultures having been prepared, one was used as a check and kept in the open room, while the other was placed in a desiccator sealed with vaseline. The use of the desiccator eliminated the water vapor which otherwise would have been present in the closed compartment. The glass capsules contained sufficient nutrient solution to keep the cultures well supplied with food and moisture throughout the experiment. Table 2 shows the results after one month.

- I. Check: cultures kept in the open room.
- II. Cultures kept in the desiccators.



TABLE 2. *The Effect of Reduced Oxygen Supply*

Organisms	I. Check	II. In Sealed Desiccator
<i>Ascochyta nymphaeae</i> .....	60	40
<i>Coniothyrium concentricum</i> .....	25	20
<i>Cytosporella mendax</i> .....	25	15
<i>Endothia parasitica</i> .....	15	15
<i>Hendersonia</i> sp.....	20	5
<i>Kellermania yuccagena</i> .....	10	10
<i>Melanconium betulinum</i> .....	20	0
<i>Naemosphaera</i> sp.....	25	25
<i>Ollula</i> sp.....	10	5
<i>Pestalozzia guepini</i> .....	20	20
<i>Phoma urens</i> .....	10	0
<i>Plenodomus des ruens</i> .....	30	15
<i>Phyllosticta opuntiae</i> .....	40	5
<i>Sphaerographium fraxini</i> .....	15	10
<i>Sphaeronema spurium</i> .....	20	5
<i>Sphaeronema pruinosum</i> .....	25	5
<i>Stagonospora collapso</i> .....	20	20
<i>Stagonospora gigantea</i> .....	10	10
<i>Steganosporium acerinum</i> .....	15	0
<i>Vermicularia circinans</i> .....	40	30

It seems that free access of air is a very important factor for pycnidium-formation in many species, although a number of others remained quite indifferent to a limited supply of oxygen.

#### Adsorption

It might be supposed that the use of filter paper would result in the adsorption by the fibers of filter paper of some of the molecules of the nutrient solution. Consequently some experiments were set up to test the adsorption effect, if any, during the development of the organisms. Shredded filter paper, powdered Jena glass, and agar agar were used. A number of glass capsules, each containing 10 cc. of the nutrient solution, were prepared and divided into four groups and treated as follows:

- I. Check: 10 cc. of nutrient solution in glass capsules.
- II. 10 cc. of nutrient solution plus 0.15 gram of agar agar.
- III. 10 cc. of nutrient solution and one gram of shredded filter paper.
- IV. 10 cc. of nutrient solution plus 15 grams of powdered Jena glass.

It is evident that adsorption of the molecules of food substances failed to be a clear-cut factor in these experiments (table 3). So long as there is a sufficient quantity of water present, adsorption phenomena can be disregarded. It was noticed, however, that, in case of cultures in which filter paper and powdered glass were used, growth ceased after the free water evaporated; in spite of the fact that the substrata were still wet, the water molecules became so strongly adsorbed, especially by the particles of the powdered glass, that the fungi were unable to obtain the necessary moisture. Similarly, when agar agar cultures lost part of their water, the adsorption became so active that the further growth of the organisms was arrested.

TABLE 3. *The Effect of Adsorption*

Organisms	I. Check		II. Plus Agar	III. Plus Filter Paper	IV. Plus Powdered Glass
	(a) On Surface of Solution	(b) Submerged			
<i>Ascochyta nymphaeae</i> .....	30	0	40	50	50
<i>Coniothyrium concentricum</i> .....	75	75	75	60	75
<i>Cytospora mendax</i> .....	25	0	75	55	75
<i>Endothia parasitica</i> .....	35	10	35	35	40
<i>Hendersonia</i> sp.....	20	10	30	50	40
<i>Kellermania yuccagena</i> .....	40	25	30	25	25
<i>Melanconium betulinum</i> .....	20	0	40	40	35
<i>Naemosphaera</i> sp.....	20	0	50	50	50
<i>Ollula</i> sp.....	25	5	30	40	45
<i>Pestalozzia guepini</i> .....	75	0	75	70	65
<i>Phoma urens</i> .....	20	5	40	40	45
<i>Plenodomus destruens</i> .....	40	70	50	40	50
<i>Phyllosticta opuntiae</i> .....	25	0	50	70	50
<i>Sphaerographium fraxini</i> .....	0	0	30	40	—
<i>Sphaeronema spurium</i> .....	10	0	15	15	15
<i>Sphaeronema pruinosum</i> .....	25	Immature	75	75	75
<i>Stagonospora collapsa</i> .....	75	0	75	80	80
<i>Stagonospora gigantea</i> .....	30	10	25	20	30
<i>Steganosporium acerinum</i> .....	25	Immature	50	40	45
<i>Vermicularia circinans</i> .....	40	Immature	50	30	60

### Transpiration

Oxidation processes play an important rôle in the reproduction of fungi, but the degree of oxygen requirement varies with the specificity of the organism. Liquids contain less available oxygen than solid substrata, consequently some organisms form no fruit bodies when submerged, while others reproduce with equal ease both above and under water. An examination of column 1b of table 3 shows that eight organisms fruited under water and matured their spores, three others formed immature pycnidia, and in the case of the remaining organisms no fruit bodies were formed under water. *Plenodomus destruens* gave rise to more fruit bodies under water than it did above the surface of the solution, while *Coniothyrium concentricum* formed pycnidia with equal ease both above and within the nutrient solution.

### Solidity of Substratum

A comparison of column 1a of table 3 with columns II, III, and IV shows that liquid media are less favorable for producing pycnidia than media which have been made more or less solid by addition of agar agar, filter paper, or powdered glass. *Coniothyrium concentricum*, *Endothia parasitica*, *Kellermania yuccagena*, *Pestalozzia guepini*, *Stagonospora collapsa*, and *Stagonospora gigantea* were the only organisms that remained unaffected by the liquid medium. The significance of this fact will be discussed later.

### Reaction of Media

*Alkalinity.* Alkalinity of the medium here used can not be considered an analyzable factor, because when enough sodium hydroxid was added to the solution to test even as high as -20 (Fuller's scale, phenolphthalein indicator), oxidation changes going on in the medium reduced the reaction of the solution to the neutral point within a few days.

*Acidity.* Since the use of hydrochloric acid, even in minute amounts, proved uniformly toxic to the organisms, tartaric acid was resorted to for testing approximate acid tolerance. Two series of standard nutrient solution<sup>1</sup> were prepared. Tartaric acid, in such concentrations as to test N/5 and N/10 acid, was added to these series. It was found that only eight organisms, namely, *Cytospora mendax*, *Endothia parasitica*, *Hendersonia* sp., *Melanconium betulinum*, *Ollula* sp., *Pestalozzia guepini*, *Plenodomus destruens*, and *Stagonospora collapsa* formed fruit bodies, and then only a few, in the more acid solution, while only four fungi, *Phyllosticta opuntiae*, *Naemosphaera* sp., *Kellermania yuccagena*, and *Stagonospora gigantea* failed to reproduce in the less acid solution. *Vermicularia circinans*, *Pestalozzia guepini*, and *Ollula* sp. were remarkably stimulated towards fruiting when grown in the less acid solution, while the rest generally produced a reduced number of fruit bodies.

### Food-concentration

#### *Historical Considerations*

Since a review of the literature on the effect of food-concentration is not as readily available as it is in the cases of the other factors, a brief summary of the more important works is given here. No one has paid so much attention to the influence of food-concentration as Klebs. In his experiments with *Hydrodictyon* (9) and other algae (10), Klebs induced the formation and discharge of zoöspores, or the development of gametes, by bringing about changes in the quantity of the available food supply of these organisms. He found that in *Saprolegnia* (12) the actual stimulus for sporangium-formation consisted of the reduction of the essential food materials to a certain minimum, and that sporangium-formation became more active with each further dilution. He states that a sudden withdrawal of food is the most favorable factor in the asexual reproduction of this organism. In the summary of his own experiments as well as those of his students and other workers, Klebs (13) cites a number of examples to substantiate his theory that "Nahrungsmangel" is a limiting factor in the reproduction of fungi. Thus, *Bacillus anthracis* multiplied vegetatively in a good food solution, while a lack of food induced sporulation. *Didymium difforme* remained in the plasmodial stage so long as it was supplied with fresh food, but when transferred to water it began to fruit. *Ascoidea rubescens* formed nothing

<sup>1</sup> See page 21 for the formula of the standard solution.

but sterile mycelium when left in plum juice, but when transferred to water, conidia developed in great abundance. *Nectria cinnabarina* yielded a vast quantity of conidia when its organic food was diminished, while the renovation of the liquid medium initiated a vigorous mycelial growth. *Pestalozzia truncatula* gave rise to a few fruit bodies on the aerial mycelium in the damp air, while on the substratum itself no pycnidia formed before the exhaustion of the food supply. According to Klebs, ascospore formation in yeasts is determined exclusively by lack of food. Guilliermond (7), summarizing the works of Klebs, Hansen, Saito, etc., concludes that ascus-formation in yeasts is a much more complex phenomenon than a mere reduction of food supply, since even the actively growing hyphae are capable of forming asci; that various food substances, and such factors as oxygen, temperature, light, humidity, acidity and alkalinity, high osmotic pressure, etc., are closely associated with ascus-formation. Raciborski (19) states that concentrated solutions checked the formation of zygospores in *Basidiobolus*. Falck (6) observed that high concentrations of a normal nutrient medium induced zygospore-formation in *Sporodinia grandis*, and that more dilute solutions favored sporangium-development. Kauffman (8), working with various species of *Saprolegnia*, verified Klebs' findings concerning the effect of nutrition upon the growth and the reproduction of these organisms. Stevens and Hall (24) observed that a very poor medium sufficed to bring about reproduction in case of a number of fungi, while richer food substances often resulted in cessation of spore-formation. Wakefield (26) found that *Schizophyllum commune* and *Stereum purpureum* could be induced to form fruit bodies by partially or wholly removing the food from the hyphae. Raybaud (21) states that when the mycelium of *Phycomyces nitens* was transferred to a high concentration of nutrient solution, after having made some growth on a medium of lower concentration, it made a good growth but formed no sporangia. When transferred to water, it gave rise to slender and well branched hyphae, but no sporangia were ever observed. This is a case in which neither a rich food nor a complete absence of food seemed to be factors in reproduction. Pieters (18) was perhaps the first investigator to determine the carrying-over properties of the mycelium. He showed that hyphae, while growing vegetatively, develop tendencies that may later affect reproduction when the fungus is subjected to the influence of changed factors. Schostakowitsch (22) found that *Fumago vegans* could be made to produce either sterile mycelium, or conidiophores without conidia, or sessile but mature fruiting bodies, merely by furnishing the fungus with media of different quality and quantity. Leininger (14) in his investigations on *Pestalozzia palmarum* found that a withdrawal of food from a growing mycelium was conducive to pycnidium-formation. Coons (4) observed that *Plenodomus fuscomaculans* produced no fruiting bodies when grown in solutions containing a high food-concentration, while on more dilute media, and even on filter paper plus conductivity water, it gave rise to pycnidia.

The effect of food-concentration upon pycnidium-formation was extensively tested in the experiments described here. The experimental results were rigidly checked and counter-checked. Whenever in doubt, the work was repeated. Some of the experiments were repeated as many as six times. As stated before, the same standard solution was used exclusively. The quality always remained the same, but the concentration was changed in each case.

*The Effect of High Food-concentrations upon Reproduction*

As a starting-point, the fungi were grown in solutions of five different concentrations of otherwise identical composition as follows:

- I. Nutrient solution of standard concentration.
- II. Twice the usual concentration.
- III. Four times the usual concentration.
- IV. Eight times the usual concentration.
- V. Sixteen times the usual concentration.

It should be noted that the standard solution<sup>1</sup> contains only 1.5 percent of food matter, while the most concentrated one contains 24 percent.

The cultures, as in all cases, were made on filter-paper pads saturated with, and partially submerged in, these various solutions.

TABLE 4. *The Effect of High Food-concentration upon Reproduction*

Organisms	I*	II	III	IV	V
<i>Ascochyta nymphaeae</i> .....	60	60	70	90	0
<i>Coniothyrium concentricum</i> .....	25	30	50	80	80
<i>Cytospora mendax</i> .....	25	50	60	85	100
<i>Endothia parasitica</i> .....	15	25	30	50	70
<i>Hendersonia</i> sp.....	20	40	45	45	50
<i>Kellermania yuccagena</i> .....	10	25	25	50	75
<i>Melanconium betulinum</i> .....	20	30	40	60	75
<i>Naemosphaera</i> sp.....	25	30	50	25	20
<i>Ollula</i> sp.....	10	20	50	65	65
<i>Pestalozzia guepini</i> .....	20	50	75	90	90
<i>Phoma urens</i> .....	10	20	30	50	60
<i>Plenodomus destruens</i> .....	30	60	70	70	80
<i>Phyllosticta opuntiae</i> .....	40	60	60	70	60
<i>Sphaerographium fraxini</i> .....	15	45	60	75	90
<i>Sphaeronema spurium</i> .....	20	40	50	75	85
<i>Sphaeronema pruinosum</i> .....	25	50	50	75	90
<i>Stagonospora collapsa</i> .....	20	40	70	90	100
<i>Stagonospora gigantea</i> .....	10	20	50	85	100
<i>Steganosporium acerinum</i> .....	15	20	30	50	60
<i>Vermicularia circinans</i> .....	40	75	75	60	60

\*See explanation above of these numerals.

It is evident that the number of pycnidia generally increases with the increase in food-concentration. Only one organism, *Ascochyta nymphaeae*, failed to form pycnidia in the highest concentration, although it did make a very rich growth. *Phyllosticta opuntiae* and *Vermicularia circinans* did

not respond very strongly to the highest concentration, while in the same solution *Naemosphaera* sp. exhibited a marked decrease in its fruiting.

In another series of experiments in which the nutrient solution used was thirty-two times the usual concentration and contained forty-eight percent of total food matter, some interesting results were obtained. Ten organisms, namely, *Phoma urens*, *Sphaeronema pruinosum*, *Plenodomus destruens*, *Cytosporella mendax*, *Kellermania yuccagena*, *Stagonospora collapsa*, *Stagonospora gigantea*, *Endothia parasitica*, *Melanconium betulinum*, and *Pestalozzia guepini*, produced a great number of fruit bodies, while the remaining organisms made a luxuriant vegetative growth but failed to form pycnidia.

#### *The Effect of a Sudden Withdrawal of Food from a Well Nourished Mycelium*

If the statement of Klebs (13), that the presence of food tends to hinder, and its sudden withdrawal from a well nourished thallus stimulates, asexual reproduction, applies not only to Phycomycetes but to Sphaeropsidales as well, then a complete withdrawal of food should promote the formation of more asexual fruit bodies than its constant presence. Consequently the following experiments were performed to test this question.

I. Check: Organisms were grown in the standard solution.

II. Check no. 2: Organisms were grown in a solution sixteen times the usual concentration.

III. The cultures were grown on pads in the usual way and in the standard solution until they formed a vigorous growth of hyphae, and then, just before the first fruit bodies appeared (this varying greatly with the specific organisms), were thoroughly washed in distilled sterile water and transferred to capsules which contained distilled sterile water.

IV. The filter-paper-pad cultures were grown submerged in the standard concentration to induce a more profuse mycelial growth and to retard fruiting. After a dense hyphal mass was obtained, the cultures were washed and transferred to capsules containing distilled sterile water.

V. The cultures were grown in the solution as in III, but with sixteen times the usual concentration, until they formed a vigorous growth of hyphae, and then, just before the first fruit bodies appeared, they were thoroughly washed in three changes of distilled sterile water, half an hour in each change, and were transferred to capsules containing distilled sterile water.

VI. The cultures were grown submerged in a solution sixteen times the usual concentration. After a dense hyphal mass was obtained, the cultures were washed in three changes of distilled sterile water and transferred to capsules containing distilled sterile water.

The results obtained with these experiments are given in table 5.

A great variety of reactions can at once be noticed. Let us first compare column III, table 5, which represents the results of a sudden withdrawal of food from a normally nourished mycelium, with column I which shows the effect of the constant presence of the standard nutrient solution. *Coniothyrium concentricum* and *Endothia parasitica* are the only organisms which showed an increase in the number of fruiting bodies as the result of the withdrawal of food. Three organisms, *Ollula* sp., *Phoma urens*, and *Pestalozzia guepini*, remained indifferent, while the rest formed fewer pycnidia.



TABLE 5. *The Effect of Sudden Withdrawal of Food*

Organisms	I*	II	III	IV	V	VI
<i>Ascochyta nymphaeae</i> .....	60	0	20	35	75	45
<i>Coniothyrium concentricum</i> .....	25	80	40	60	80	80
<i>Cytosporella mendax</i> .....	25	100	20	50	60	60
<i>Endothia parasitica</i> .....	15	70	20	50	70	70
<i>Hendersonia</i> sp.....	20	50	10	20	30	70
<i>Kellermania yuccagena</i> .....	10	75	5	5	40	40
<i>Melanconium betulinum</i> .....	20	75	15	20	25	25
<i>Naemosphaera</i> sp.....	25	20	15	35	60	60
<i>Ollula</i> sp.....	10	65	10	20	65	40
<i>Pestalozzia guepini</i> .....	20	90	20	35	75	75
<i>Phoma urens</i> .....	10	60	10	20	10	20
<i>Plenodomus destruens</i> .....	30	80	20	25	65	80
<i>Phyllosticta opuntiae</i> .....	40	60	25	95	60	95
<i>Sphaerographium fraxini</i> .....	15	90	5	20	20	20
<i>Sphaeronema spurium</i> .....	20	85	10	15	50	40
<i>Sphaeronema pruinosum</i> .....	25	90	15	50	50	70
<i>Stagonospora collapsa</i> .....	20	100	15	25	30	25
<i>Stagonospora gigantea</i> .....	10	100	0	25	60	50
<i>Steganosporium acerinum</i> .....	15	60	5	5	20	20
<i>Vermicularia circinans</i> .....	40	60	20	35	85	35

\* See explanation above of these numerals.

When the cultures were grown submerged in the standard concentration (column IV), they were able to lead a longer vegetative life, had a larger quantity of food available, accumulated a higher degree of morphogenic energy, and consequently gave rise to more fruiting bodies. *Ascochyta nymphaeae*, *Steganosporium acerinum*, *Kellermania yuccagena*, *Plenodomus destruens*, *Sphaeronema spurium*, and *Vermicularia circinans* showed a slight reduction in the number of their fruit bodies; *Hendersonia* sp. and *Melanconium betulinum* were unaffected, while the remaining organisms were favorably stimulated.

Passing now to the highest concentration, let us compare column II which shows the effect of the constant presence of much food throughout the life cycle of these fungi, with column V in which are summarized the results obtained by the sudden withdrawal of food from an actively growing mycelium. *Ascochyta nymphaeae*, *Naemosphaera* sp., and *Vermicularia circinans* are the only organisms which formed more fruit bodies when the food was withdrawn than when it was constantly present. However, it should not be forgotten that the first-named organism does not form pycnidia at all when left growing in the high concentration, and the remaining two do not attain their maximum reproduction when the concentration is as high as this. *Coniothyrium concentricum*, *Endothia parasitica*, *Ollula* sp., and *Phyllosticta opuntiae* remained unaffected, while all the other organisms, thirteen in number, formed a reduced number of fruit bodies as a result of the withdrawal of the rich food from the mycelium.

When the cultures were grown submerged in the high concentration and then washed and transferred to distilled water, only five organisms, namely,

*Hendersonia* sp., *Phoma urens*, *Plenodomus destruens*, *Phyllosticta opuntiae*, and *Sphaeronema pruinosum*, exhibited an increase in their fruit bodies. *Ascochyta nymphaeae*, *Ollula* sp., *Sphaeronema spurium*, *Stagonospora collapsa*, *Stagonospora gigantea*, and *Vermicularia circinans* had a decreased reproduction, while the remaining nine organisms were indifferent in their reactions.

*Cytospora mendax* showed some interesting modifications: after making a good vegetative growth, and after being washed and transferred to distilled water, it failed to form stromata, and most of the fruiting appeared as naked spore masses, the pycnidial walls of which were entirely eliminated. *Endothia parasitica* under similar treatment also formed no stromata, and pycnidia likewise appeared singly.

Table 5 shows that a number of organisms produced very few fruit bodies when they were transferred from either the high or the low concentration of the nutrient solution directly to distilled water. In order to find if the mycelium still retained its normal vitality in spite of its inability to reproduce well, the cultures were retransferred from distilled water to the nutrient solution of standard concentration. Shortly after the transfer, a very large number of fruit bodies appeared, in some cases covering every available space of the substratum with pycnidia. Apparently a transfer to distilled water initiates a great number of the microscopic beginnings of pycnidial stages, but few of these mature unless sufficient food is present.

An experiment was set up to find whether there was a direct parallel between the quantity of mycelium and the amount of pycnidia formed. Cultures were started in the standard solution. After a fair hyphal growth was obtained and before the first fruit bodies appeared, the cultures were transferred to capsules each of which contained 20 cc. of the standard solution. The organisms were allowed to grow submerged in this solution. Then the cultures were divided into three groups: the first was allowed to grow submerged for two days; the second, for four days; and the third, for eight days. At the end of these periods of growth the cultures were washed and transferred to distilled sterile water. In every case the quantity of hyphae was proportionally increased with the length of the period in the submerged condition. In only four organisms could an appreciable proportional difference be seen between the quantity of the mycelium and the amount of fruit bodies. *Sphaerographium fraxini*, *Sphaeronema pruinosum*, and *Stagonospora gigantea* formed a larger number of pycnidia as the length of the vegetative period was increased; *Phyllosticta opuntiae* exhibited a decrease in its number of pycnidia after it was allowed to grow submerged for eight days and then transferred to distilled water; however, very good development of pycnidia was obtained when the submerged growth was reduced from eight to four days. In the case of the remaining sixteen organisms the number of fruit bodies was the same in a given area, regardless of the quantity of mycelium.



*The Effect of Gradual Changes of Concentration*

We have seen that a sudden withdrawal of food induces three types of reactions depending on the fungi worked with: some are stimulated to better pycnidial production, others remain indifferent, while still others exhibit a more or less decided decrease in the quantity of fruiting bodies. The next logical step in these investigations was to test, first, the effect of a gradual withdrawal of food until a known minimum is reached, and second, to find if a gradual increase of concentration to a known maximum and then a sudden transfer to a known minimum would induce reactions similar to those recorded in column V of table 5. The five concentrations of the nutrient solutions, as previously described, were used in the following experiments.

Check: The organisms were grown in the standard solutions throughout their life cycle.

I. The organisms were grown on filter-paper pads and kept in the standard solution; after a good hyphal growth had been obtained and just before the first fruiting bodies had appeared, the cultures were washed in distilled sterile water and transferred to another series of solutions of twice the usual concentration. Twenty-four hours later the cultures were washed and transferred to solutions of four times the usual concentration, and the process was continued in this way until solutions of sixteen times the usual concentration were reached; in the latter solutions the cultures were allowed to remain for a day, after which they were washed and transferred to solutions of the standard concentration.

II. The reverse of the above-described treatment: The organisms in this case were grown on filter-paper pads kept in solutions of standard concentration until a good hyphal growth was obtained: then they were washed and transferred directly to solutions of sixteen times the usual concentration. At regular intervals of twenty-four hours they were gradually passed down to the less and less concentrated solutions until the standard solution was reached.

III. In this series, after the cultures had made a good vegetative growth in the standard solutions, they were washed and transferred to a fresh lot of the standard solution. This process was repeated for five days at twenty-four hour intervals, when the cultures were left undisturbed.

IV. Same as in III, except that washing operations before each transfer were eliminated.

The last two experiments eliminate osmotic pressure as a possible factor, and do away with the objection that the process of washing the cultures free from the accumulated waste matter may be the real cause of the results obtained.

In most cases it makes little or no difference whether a normally grown mycelium is gradually transferred from a low to a higher concentration, or whether it is transferred directly to the high concentration and is gradually carried down to the lower one. *Plenodomus destruens*, *Cytospora mendax*, *Naemosphaera* sp., *Kellermania yuccagena*, *Stagonospora gigantea*, *Hendersonia* sp., *Steganosporium acerinum*, *Sphaerographium fraxini*, *Endothia parasitica*, *Melanconium betulinum*, and *Pestalozzia guepini* showed an increased pycnidium-production when transferred from lower to higher

TABLE 6. *The Effect of Gradual Increase or Decrease of Food-concentration*

Organisms	Check	I*	II	III	IV
<i>Ascochyta nymphaeae</i> .....	60	65	65	20	70
<i>Coniothyrium concentricum</i> .....	25	100	100	70	70
<i>Cytospora mendax</i> .....	25	85	60	60	70
<i>Endothia parasitica</i> .....	15	75	70	50	40
<i>Hendersonia</i> sp.....	20	90	80	35	30
<i>Kellermania yuccagena</i> .....	10	20	10	5	30
<i>Melanconium betulinum</i> .....	20	60	50	40	40
<i>Naemosphaera</i> sp.....	25	60	50	60	60
<i>Ollula</i> sp.....	10	50	50	30	40
<i>Pestalozzia guepini</i> .....	20	100	90	65	75
<i>Phoma urens</i> .....	10	50	50	50	50
<i>Plenodomus destruens</i> .....	30	90	80	80	90
<i>Phyllosticta opuntiae</i> .....	40	50	70	80	60
<i>Sphaerographium fraxini</i> .....	10	40	80	15	15
<i>Sphaeronema spurium</i> .....	20	80	50	30	30
<i>Sphaeronema pruinosum</i> .....	25	90	90	40	40
<i>Stagonospora collapsa</i> .....	20	85	85	75	75
<i>Stagonospora gigantea</i> .....	10	90	80	45	60
<i>Steganosporium acerinum</i> .....	15	100	85	20	20
<i>Vermicularia circinans</i> .....	40	100	100	100	100

\* See explanation above of these numerals.

concentrations. In the case of *Phyllosticta opuntiae*, pycnidium-formation was reduced and the remaining organisms were indifferent. When compared with the checks, however, a general and sharp increase in pycnidium-production was noticeable for all species.

A comparison of the fourth column of table 6 with the second column shows that in the cases of some organisms it makes no difference whether the concentration of the solution is gradually increased, or whether it is kept at the same low concentration, provided that a fresh solution is used once a day for five consecutive days. *Phyllosticta opuntiae* formed more fruit bodies when it was given a fresh solution of low concentration for five days than it did when higher concentrations were used. *Naemosphaera* sp., *Phoma urens*, and *Vermicularia circinans* were indifferent, while the fruiting was more or less reduced in the remaining organisms.

*Phyllosticta opuntiae* and *Naemosphaera* sp. formed more pycnidia when they were washed and transferred to a fresh supply of the standard concentration for five consecutive days than when transferred to the standard medium gradually from the solution of sixteen times the usual concentration. *Cytospora mendax*, *Phoma urens*, *Plenodomus destruens*, and *Vermicularia circinans* remained indifferent, while the remaining organisms gave rise to fewer fruit bodies.

The elimination of washing operations before each transfer not only failed to be harmful, but it became decidedly beneficial for some organisms, as can be seen in the last column of table 6. *Ascochyta nymphaeae*, *Cytospora mendax*, *Kellermania yuccagena*, *Ollula* sp., *Pestalozzia guepini*, *Plenodomus destruens*, and *Stagonospora gigantea* showed an increased

pycnidium-production. Only *Endothia parasitica*, *Hendersonia* sp., and *Phyllosticta opuntiae* showed a reduced quantity of pycnidia, while the remaining species were indifferent.

It can be seen that six organisms, namely, *Hendersonia* sp., *Steganosporium acerinum*, *Melanconium betulinum*, *Sphaerographium fraxini*, *Sphaeronema spurium*, and *Sphaeronema pruinosum*, failed to respond very vigorously when they were carried through five changes of the standard solution. It was concluded that a longer growing period might be responsible for this behavior, and that, if the transfer were made at longer intervals, the organisms might be able to utilize the fresh supply of the nutrient solution to better advantage. Consequently, in an additional series, the first transfer was made after the appearance of the fruit bodies. Shortly thereafter a vigorous reproduction took place, so that, with the exception of *Sphaerographium fraxini*, all other organisms showed a remarkable gain in the quantity of pycnidia produced.

It should be stated here that washing operations caused an abundant development of hyphomycetous conidia in cultures of *Ascochyta nymphaeae*. It seems that this organism tends to behave in this way regularly when washed in distilled water. When the cultures of this species were gradually transferred from high to low or from low to high concentrations, an abnormally large quantity of hyphomycetous conidia appeared and covered the substratum with a solid mass of salmon-colored spores; later, pycnidia appeared and covered this spore mass. *Cytospora mendax* showed a similar, but not always so prominent, tendency.

That a fresh supply of food of the standard concentration, supplied at regular intervals to the growing organisms, brings about results similar to those which were obtained with the higher concentrations of the same solution, becomes apparent by comparing the first column of table 6 with the last two columns. With the exception of *Sphaerographium fraxini*, which remained indifferent, and which has given erratic results in many of the experiments, all the organisms showed an increase in the number of pycnidia. *Kellermania yuccagena* showed a reduced pycnidial production when the cultures were washed before being transferred to a fresh solution. This has been the case with this particular organism every time that it was washed in distilled water. However, it never has been a very vigorous and free pycnidium-former, at least not in the medium used. Although *Ascochyta nymphaeae* showed a decrease in number of pycnidia as a result of washing the cultures in distilled water, it gave rise, nevertheless, to a much larger quantity of hyphomycetous conidia and thus made up for the deficiency in the number of pycnidia.

#### *The Effect of Sudden Increase of Concentration on Normally Grown Mycelium*

After testing the effects of *sudden withdrawal* of food and of *gradual increase or decrease* in food-concentration, it was decided to determine the

effect of a sudden increase in food-concentration. Consequently the following experiment was set up.

I. Check: The organisms were grown on filter-paper pads kept in solutions of sixteen times the standard concentration.

II. After the organisms had made a good growth on filter-paper pads kept in the standard concentration, and before the first visible fruit bodies had appeared, they were washed and transferred to solutions of sixteen times the standard concentration.

TABLE 7. *Effect of a Sudden Increase of Concentration upon a Normally Grown Mycelium*

Organisms	I. Check	II. Transferred to High Concentration
<i>Ascochyta nymphaeae</i> .....	0	100
<i>Coniothyrium concentricum</i> .....	80	90
<i>Cytospora mendax</i> .....	100	100
<i>Endothia parasitica</i> .....	70	50
<i>Hendersonia</i> sp.....	50	100
<i>Kellermania yuccagena</i> .....	75	75
<i>Melanconium betulinum</i> .....	75	100
<i>Naemosphaera</i> sp.....	20	10
<i>Ollula</i> sp.....	65	25
<i>Pestalozzia guepini</i> .....	90	100
<i>Phoma urens</i> .....	60	85
<i>Plenodomus destruens</i> .....	80	100
<i>Phyllosticta opuntiae</i> .....	60	40
<i>Sphaerographium fraxini</i> .....	90	100
<i>Sphaeronema spurium</i> .....	85	100
<i>Sphaeronema pruinosum</i> .....	90	100
<i>Stagonospora collapsa</i> .....	100	100
<i>Stagonospora gigantea</i> .....	100	100
<i>Steganosporium acerinum</i> .....	60	100
<i>Vermicularia circinans</i> .....	60	85

In the cases of *Endothia parasitica*, *Naemosphaera* sp., *Ollula* sp., and *Phyllosticta opuntiae*, the fruiting was reduced as a result of sudden transfer to a food of high concentration. *Kellermania yuccagena* remained indifferent, while all the other organisms exhibited a remarkable increase in the quantity of pycnidia. *Ascochyta nymphaeae*, which failed to form any pycnidia when kept constantly in solutions of high concentration, developed a large number of fruit bodies when transferred to such a solution in the manner described. It might, perhaps, be supposed that the filter-paper pad, when being washed in distilled water, would absorb enough water to dilute the nutrient solution to which it was transferred. Further experiments showed, however, that this was not a factor in the case. For when the cultures, after being washed in distilled water, were allowed to evaporate to complete dryness and then transferred directly to solutions of sixteen times the standard concentration, they produced pycnidia just as abundantly. The small amount of water that might be carried into the new solution by the wet filter paper soon evaporates, and the culture method is such that the fungus utilizes all or most of the nutrient matter by the time it completes its life cycle. *Stagonospora collapsa*, which normally formed pycnidia singly, gave rise to a number of large, well developed stromata when it was transferred to solutions of high food concentration.

*The Effect of Food of the Standard Concentration upon Mycelium Richly Fed and then Allowed to Starve*

Klebs (10, 12, 13) states that the sudden removal of food stimulated asexual reproduction in a number of organisms with which he and his students experimented. Kauffman (8), Pieters (18), and others have verified Klebs' findings in Saprolegnias and other organisms. Yet we have seen (p. 31) that, in the cases of a large number of organisms worked with in the present investigation, a sudden withdrawal of food from a well nourished mycelium failed to induce the formation of pycnidia to the same extent as when food was constantly present. It was therefore assumed that these organisms require a longer period for the production of these bodies and need a longer-continued food supply: and that, although the sudden removal of food did not stimulate the fungus sufficiently to initiate and mature a large number of pycnidia, it might, nevertheless, be effective to the extent that it might initiate the beginnings of a larger number of fruit bodies and that these might have remained undeveloped because of the absence of food. If this hypothesis is correct, then the mycelium, after a period of starvation, should give rise to a large number of pycnidia when transferred to the standard solution. Accordingly, the following experiments were set up.

I. Check: Cultures were made and kept in solutions of sixteen times the usual concentration.

II. Check no. 2: Cultures were made and kept in solutions of sixteen times the usual concentration until a good hyphal growth was obtained and before the first fruit bodies appeared; then they were washed in several changes of distilled water and transferred to distilled water.

III. Cultures were made and kept in solutions of sixteen times the usual concentration until a good hyphal growth was obtained and before the first fruit bodies appeared; then they were washed thoroughly in three changes of water, one half hour in each change, and transferred to the standard solution.

IV. Same as experiment III, except that instead of transferring the cultures directly to the ordinary nutrient solution they were first kept in distilled sterile water until they gave rise to as many fruit bodies as possible; then they were transferred to the standard solution.

Table 8 gives the results.

The difference between the third and the fourth columns of table 8 is so slight as to be disregarded altogether except in case of the figures for *Phyllosticta opuntiae*. The optimum food-concentration for this organism seems to be very low, so that even the standard solution, when immediately furnished to the richly nourished mycelium, is sufficient to disturb the equilibrium between vegetative growth and reproduction at the expense of the pycnidia. A comparison between the last two columns and the second column shows that a period of starvation initiates a larger quantity of fruiting bodies which can develop and reach maturity if furnished with a smaller amount of food. *Hendersonia* sp., *Steganosporium acerinum*,

TABLE 8. *The Effect of a Weak Food Solution Upon a Mycelium After It Was Richly Fed and Then Starved*

Organisms	I*	II	III	IV
<i>Ascochyta nymphaeae</i> .....	0	70	70	70
<i>Coniothyrium concentricum</i> .....	80	80	80	80
<i>Cytospora mendax</i> .....	100	60	60	60
<i>Endothia parasitica</i> .....	70	70	65	70
<i>Hendersonia</i> sp. ....	50	30	40	40
<i>Kellermania yuccagena</i> .....	75	40	60	60
<i>Melanconium betulinum</i> .....	75	25	40	40
<i>Naemosphaera</i> sp. ....	20	60	75	75
<i>Ollula</i> sp. ....	65	65	65	65
<i>Pestalozzia guepini</i> .....	90	75	75	80
<i>Phoma urens</i> .....	60	10	45	50
<i>Plenodomus destruens</i> .....	80	65	80	80
<i>Phyllosticta opuntiae</i> .....	60	60	40	85
<i>Sphaerographium fraxini</i> .....	90	20	35	35
<i>Sphaeronema spurium</i> .....	85	50	55	60
<i>Sphaeronema pruinosum</i> .....	90	50	50	50
<i>Stagonospora collapsa</i> .....	100	30	70	70
<i>Stagonospora gigantea</i> .....	100	60	75	75
<i>Steganosporium acerinum</i> .....	60	20	45	45
<i>Vermicularia circinans</i> .....	60	85	85	85

\* See explanation above of these numerals.

*Kellermania yuccagena*, *Melanconium betulinum*, *Naemosphaera* sp., *Phoma urens*, *Pestalozzia guepini*, *Plenodomus destruens*, *Phyllosticta opuntiae*, *Sphaerographium fraxini*, *Sphaeronema spurium*, *Stagonospora collapsa*, and *Stagonospora gigantea* were stimulated and produced more pycnidia, while the remaining species were indifferent. It appears that the dilute nutrient solution may not only act like distilled water in initiating a larger number of initial pycnidia, but that it may also furnish them with enough food for their maturing.

### The Carrying-over Effect of the Mycelium

Many of the experiments already described have shown that the mycelium may acquire a certain tendency when subjected to one set of environmental factors and may manifest no sign of this tendency unless it is acted upon by a changed environment. Klebs (12, 13) recognized similar phenomena, and Pieters (18) demonstrated them more definitely. In order to demonstrate this carrying-over effect of the mycelium more strikingly, it was necessary to employ a different nutrient solution from the one used throughout these experiments; a solution that would prove uniformly hostile to pycnidium-formation in all the organisms. The following formula was found to give the most satisfactory result in this respect: ammonium nitrate 1 gram, dihydrogen potassium phosphate 0.5 gram, magnesium sulphate 0.25 gram, cane sugar 5 grams, and distilled water 100 cc. For the sake of convenience, this will be referred to as solution *B*, while the standard

one used throughout the work will be termed solution *A*. The following experiments were set up.

- I. Check no. 1: Organisms were grown in solution *A*.
- II. Check no. 2: Organisms were grown in solution *B*.
- III. Check no. 3: Organisms were grown in solution *B* until a good hyphal growth was obtained; then they were washed in distilled sterile water and transferred to a fresh supply of solution *B*.
- IV. Organisms were grown in solution *B* until a good hyphal growth was obtained; then they were thoroughly washed in distilled sterile water and transferred to capsules containing distilled sterile water.
- V. Organisms were grown in solution *B* until a good hyphal growth was obtained; then they were thoroughly washed and transferred to solution *A*.
- VI. Organisms were grown in solution *A* until a good hyphal growth was made; then they were washed and transferred to solution *B*.

TABLE 9. *The Carrying-over Effect of the Mycelium*

Organisms	I*	II	III	IV	V	VI
<i>Ascochyta nymphaeae</i> .....	60	0	0	5	25	5
<i>Coniothyrium concentricum</i> .....	25	0	0	0	80	30
<i>Cytospora mendax</i> .....	25	0	0	0	25	5
<i>Endothia parasitica</i> .....	15	5	5	5	35	5
<i>Hendersonia</i> sp.....	20	0	0	0	60	15
<i>Kellermania yuccagena</i> .....	10	0	0	0	10	5
<i>Melanconium betulinum</i> .....	20	0	0	0	20	0
<i>Naemosphaera</i> sp.....	25	0	0	0	40	0
<i>Ollula</i> sp.....	10	0	0	0	15	10
<i>Pestalozzia guepini</i> .....	20	0	0	20	80	20
<i>Phoma urens</i> .....	10	0	0	5	20	0
<i>Plenodomus destruens</i> .....	30	5	5	10	20	60
<i>Phyllosticta opuntiae</i> .....	40	0	0	0	30	0
<i>Sphaerographium fraxini</i> .....	15	0	0	0	35	0
<i>Sphaeronema spurium</i> .....	20	5	5	0	40	25
<i>Sphaeronema pruinosum</i> .....	25	0	0	0	20	0
<i>Stagonospora collapsa</i> .....	20	100	100	25	100	100
<i>Stagonospora gigantea</i> .....	10	5	5	5	40	5
<i>Steganosporium acerinum</i> .....	15	0	0	0	20	5
<i>Vermicularia circinans</i> .....	40	70	85	40	70	100

Table 9 shows that solution *A* induced the formation of fruit bodies in all of the twenty organisms, while solution *B* inhibited it in fourteen. Four of the remaining six fungi developed but few pycnidia, while *Stagonospora collapsa* and *Vermicularia circinans* gave rise to a much larger quantity of fruit bodies than they did when grown in solution *A*. Column III, which represents the results obtained when the cultures were transferred from solution *B* to a fresh supply of the same solution, is very much like column II, except that *Vermicularia circinans* formed more fruit bodies as a result of the transfer. When transferred from solution *B* to distilled water, *Pestalozzia guepini* and *Phoma urens* developed pycnidia, the former organism giving rise to as many of them as it did when grown in solution *A*. The most

\* See explanation above of these numerals.

remarkable results, however, are to be observed in column V. After a transfer from solution *B* to solution *A*, only four organisms, namely, *Ascochyta nymphaeae*, *Plenodomus destruens*, *Phyllosticta opuntiae*, and *Sphaeronema pruinosum*, exhibited a reduced amount of fruiting. Three others, *Cytospora mendax*, *Kellermania yuccagena*, and *Melanconium betulinum*, were unaffected by the change, while all the remaining organisms were stimulated so as to produce more pycnidia. If we now turn our attention to the last column, where the order was reversed—that is, the organisms were first grown in solution *A* and then transferred to solution *B*—we see that *Plenodomus destruens* and *Vermicularia circinans* gave rise to a still better pycnidial production. However, *Melanconium betulinum*, *Naemosphaera* sp., *Phoma urens*, *Sphaerographium fraxini*, and *Sphaeronema pruinosum* failed to fruit, *Stagonospora collapsa* was unaffected, while the remaining organisms showed a reduction in quantity of fruit bodies.

#### DISCUSSION

*Light.* It has been seen (table 1) that light is an important factor in the fruiting of a number of organisms. The intensity of action of this stimulus, however, varies with the specific nature of each fungus, or with the altered combinations of other stimuli. Thus, although *Sphaerographium fraxini* formed no pycnidia in the dark and at room temperature, it did give rise to a few fruit bodies at a temperature of 30° C. Other organisms, which were not able to develop more than very few pycnidia in the dark and at room temperature, or in the ordinary nutrient solution, gave rise to a greater abundance of fruit bodies when the temperature was raised, or when the concentration of the medium was increased, even though light was excluded. It may be said (Coons, 4, p. 761) that the direct result of the presence of light is oxidation; that, since a higher temperature or a richer food tends to bring about a higher oxidation and a more rapid metabolism, the increased reproduction would naturally be traced to the effect of oxidation. But it should not be forgotten that *Hendersonia* sp. failed to give rise to a single fruit body either when growing in a higher concentration of food, or at a higher temperature so long as light was excluded. Perhaps a higher oxidation requirement, coupled, on the one hand, with a possible inhibiting action of the higher temperature, and, on the other hand, with the less favorable influence of a higher food-concentration, altered the balance between the mycelium and the fruit bodies in favor of hyphal development and culminated in the sterility of the cultures. While this explanation sounds plausible, we have the experiments of Lendner (15) in which a mere addition of agar agar to a nutrient solution supplemented the white light so that *Mucor flavidus* was able to form sporangia in spite of the absence of white light.

*Temperature.* As has already been stated, a higher temperature is not only capable of inducing the development of more pycnidia, but in



some cases it even replaces, partially or wholly, the effect of light (table 1, column III). The majority of the organisms either failed to fruit at 8° C., or pycnidium-formation was materially reduced. The eight organisms which formed no pycnidia at this low temperature and in the dark gave rise, however, to a normal number of fruit bodies when they were transferred to the light but were still kept at the same temperature. The low temperature, provided that light is not excluded, merely delays fruiting. A slow oxidation and a slow metabolism seem to be responsible for this delay. But when light is excluded, oxidation falls below the minimum requirement for pycnidium-formation, but not below that for growth; consequently the organisms lead a sterile life until they are transferred to light.

*Oxygen Supply.* The works of Raulin (20), Schostakowitsch (22), Klebs (10, 11), Coons (4), etc., as well as the experiments outlined in this paper (table 2), show that a lack of oxygen supply is generally an inhibiting factor in the growth and reproduction of a large number of fungi. Only six organisms out of the twenty worked with remained unaffected by a reduced oxygen supply. Perhaps a narrower oxygen requirement on the one hand, and a much shorter life cycle on the other, were the chief factors responsible for the behavior of these six organisms.

*Adsorption.* Adsorption of nutrient salts appears not to be a factor in the growth and the reproduction of the organisms so long as there is a good supply of moisture (table 3). The adsorption of water molecules, however, is an important factor. We have seen that the organisms stopped growth when the moisture was decreased, and when the fibers of filter paper, the molecules of the colloid (agar agar), or the particles of powdered glass held the water molecules so firmly that the hyphae were powerless to utilize them. In a former paper (16) the writer demonstrated that *Fusarium annuum*, when grown on different types of soil, was unable to make further growth after the moisture content of a very fine sand substratum was reduced to 5.6 percent, while in clay-soil substrata growth ceased in spite of the fact that the soil still contained 10.5 percent of capillary moisture. The smaller particles of clay soil with their larger adsorbing surfaces were evidently responsible for this result. It follows that there is a very strong affinity between the water molecules and the non-living, non-assimilative particles. It is also probable that in culture media there is a certain amount of adsorption of salts, but not in large enough quantities to have an effect on the development of the organisms. Filter-paper fibers have an immense adsorbing area, and agar agar molecules are known to have such a powerful adsorbing capacity that even one percent agar agar can adsorb and solidify under certain conditions ninety-nine percent of water; yet fungi have no difficulty in obtaining their nourishment in spite of the presence of these adsorbing agents.

*Transpiration.* Not all of the organisms were affected by a lack of free transpiration. We have seen (table 3) that the fungi in their relation to

this factor can be divided into five groups: first, those which must be surrounded in part by air in order to be able to form pycnidia; second, those which form fruit bodies either submerged or on top of the nutrient solution, but more abundantly when surrounded by air; third, those which give rise to more pycnidia when submerged than they do on the surface of the solution; fourth, those which reproduce with equal ease under any of these conditions; and fifth, those organisms which are capable of forming pycnidia when submerged, but which must rise above the water in order to mature their spores. Oxygen requirements and transpiration are largely responsible for these variations. Reproduction under water, however, is affected not only by chemical, but by physical factors as well. This matter is discussed more thoroughly in the following paragraph.

*Solidity of Substratum.* An examination of the first column of table 3 leads one to wonder why so many organisms failed to do well in liquid media. Since experiments outlined and tabulated in the same table have indicated that adsorption is not a factor so long as there is an ample supply of available moisture, and since tables 4, 5, 6, and 7 show that higher food-concentrations favor rather than hinder reproduction, it can not be asserted that the presence of agar agar or filter paper in the nutrient solution tends to adsorb the molecules of food substance, and so leaves a poorer medium which discourages growth and stimulates pycnidium-formation. A reduced air supply in a liquid medium coupled with a total lack of transpiration on the one hand, and the absence of adequate supporters, on the other hand, to keep buoyant the hyphae of such organisms as always tend to form a very compact and heavy mycelium, may be largely responsible for the reduced production of pycnidia. Those fungi which naturally form a light and floccose mycelium will find sufficient support in the solution to keep them floating and to induce the development of good aërial hyphae which, because of a free transpiration, are not hindered from giving rise to fruiting bodies. Such slow-growing organisms as *Sphaeronema spurium* and *Sphaerographium fraxini*, which produce a very heavy and compact mycelial mass, sink into the solution and grow submerged, and are thus unable to form pycnidia. Solid media offer a more available air supply and allow a freer oxidation and respiration as well as a better support for the free ramification of hyphal threads. Negative adsorption whereby the water molecules are adsorbed instead of the salts, thus bringing about a more concentrated solution, may also be a factor in this case.

*Food-concentration.* Almost all the important investigations concerning the influence of food-concentration have been made with the Phycomycetes. Since there is a wide morphological dissimilarity between the Phycomycetes and the Sphaeropsidales, might it not be likely that the physiological differences may prove to be just as distinct? The Phycomycetes are coenocytic, the entire mycelium being one continuous tube system which enables the protoplasm to respond or migrate from one end of the mycelium

to the other under the stimuli which govern reproduction (*Conidiobolus utriculosus*; Brefeld, 3). DeVries (5) and others have observed the movement of the protoplasm in *Phycomyces* into the newly forming sporangia. Arthur (2) examined protoplasmic movements in a number of *Mucoraceae* and observed that hyphae, microsomes, food bodies, nuclei, and vacuoles participate. He states that the flow of protoplasm is most commonly towards the free aërial parts and the fast growing tips where a rapid use of food removes obstacles from the advancing column of protoplasm. This movement was seen to come to a halt whenever a cross wall appeared. We can see, therefore, that a heavy demand made by the sporangiophores upon the rest of the mycelium for a large quantity of food can be speedily met by the migration of the protoplasm into the sporangium-initials.

It has been shown that the constant presence of a specific type of rich food breaks up the balance between vegetative growth and asexual reproduction, stimulating the former and suppressing the latter; hence the sterility of certain species of *Saprolegnia* when grown in pea broth (Kauffman, 8; Pieters, 18). On the other hand, sexual reproduction demands comparatively more food than sporangium-formation, and therefore a total absence of food hinders the development of oögonia. Klebs (12, 13) states that the minimum concentration of the most important food materials is higher for oöspore formation than it is for zoöspore development.

Now, if we consider the *Sphaeropsidales*, we find that the septate nature of the hyphae prevents an appreciable migration of the protoplasm from cell to cell and hinders an adequate translocation of food into the pycnidium-initials. The osmotic transference is presumably much slower than in the *Phycomycetes*. This is especially true in the case of those organisms which form stromata, thick pycnidial walls, and large spores rich in reserve food. Consequently, most of the *Sphaeropsidales*, when allowed to starve, even though they have been grown in media rich in food, tend to form fewer fruit bodies than they do when food is constantly present. *Phyllosticta opuntiae* and similar organisms which form very small pycnidia and spores, the latter poor in reserve food, naturally do not require a very rich food supply, and form fruit bodies best when this supply is withdrawn from the actively growing mycelium.

Higher concentrations of the nutrient solution induce the formation of more mycelium and more pycnidia until a given maximum is reached. In other words, mycelial growth and reproduction are parallel within a very wide margin. Coons (4) states that *Plenodomus fuscomaculans* when grown in rich nutrient solutions forms its pycnidia not upon the substratum itself, but on the aërial strands, and he explains this by saying that a dense mycelial mat formed over the agar effectively walls off the new food supply, and that "with the increase in concentration in the medium below and the drying of the threads, the diffusion of food stuffs to the aërial parts is interfered with." The lack of food thus brought about is said to become responsible for the

appearance of the pycnidia on the aërial strands of his fungus. But it can not be assumed that in the experiments shown in table 4 any "walling off" effect could be operative. In no case were the pycnidia formed on wefts of aërial mycelium, but they were directly seated upon or imbedded in the filter-paper pads which were saturated with, and partially submerged in, the nutrient solution; thus, young or old fruit bodies were always in direct contact with the food. *Ascochyta nymphaeae* formed a great abundance of aërial hyphae in the richest nutrient solution, but not a single pycnidium. Similarly, on a nutrient medium which contained forty-eight percent of available food, nine other organisms made an unusually rich aërial growth but gave rise to no fruit bodies.

Osmotic pressure can not be a factor in the fruiting of the organisms worked with in these experiments. The last column of table 6 shows that it makes little or no difference in these organisms whether their demand for more food is supplied all at once in the form of a high concentration of nutrient solution or whether the food is supplied bit by bit in the form of a fresh supply offered as a weak nutrient medium (table 6, columns III and IV). In the first case there is a high osmotic pressure, while in the second case this pressure, so far as the solution itself is concerned, remains constant and very weak; yet pycnidium-production is increased most remarkably. In both cases it is the same factor that functions, namely, the large supply of food. Apparently the quantity makes up for the concentration. But, it may be asked, how is it that five changes of a solution which is so dilute in its food value are as effective as a solution sixteen times the usual concentration? At most the organisms have access to a total of food which is only five times as strong as the standard concentration, yet the effect is much greater for the production of pycnidia. It is not probable that fungi exhaust all available food supply when grown in a nutrient solution, because a cessation of growth does not necessarily mean a lack of food, but may signify that the food-absorbing capacity of the mycelium has reached its limit so far as a given solution is concerned. When grown in a nutrient solution of high concentration, these organisms sooner or later reach the limit of their absorbing capacity, and therefore utilize only a part of the available food. Probably the five transfers into a fresh medium of low concentration furnished as much food as the solution of high food-concentration. This, naturally, led to a better reproducing capacity in the majority of the fungi. It should also be remembered that the cultures were first transferred to a new solution after they had made a good growth, and had, therefore, in addition to a well developed vigor and distinct physiological changes towards pycnidium-production, a very extensive absorbing system capable of taking in and assimilating within a very short time large quantities of food from the fresh supply of nutrient solution. During the five consecutive transfers the constantly growing mycelium developed a still larger absorbing surface and stored away much food. Thus there was a constant ac-

cumulation of nutrient substances in the protoplasm; naturally this provided the basis for a high efficiency in the production of pycnidia, whereas those cultures which were kept constantly in the same supply of dilute food soon exhausted their absorbing capacity and did not have sufficient food for an abundant reproduction. But not all the organisms behaved similarly. Perhaps some of them could not get the maximum amount of necessary food from such a large quantity of dilute nutrient solution because of an innate slow growth and absorption. This hypothesis is strengthened by the fact that when these organisms were transferred, after they had ceased their growth and pycnidium-formation in the standard concentration, to a fresh supply of the same solution, the number of pycnidia increased very remarkably. An organism which has a short life cycle derives the most benefit when it is transferred to five changes of dilute nutrient medium for five consecutive days, whereas organisms with much longer life cycles and less rapid growth form comparatively fewer hyphae in a period of five days, absorb and store away less food, and as a result show only a slight or no increase in production of fruit bodies.

In contrast to the general rule in the Phycomycetes as to asexual reproduction (Klebs, 13; Kauffman, 8; Pieters, 18), we find that in most of the Sphaeropsidales a vigorous growth of mycelium and a good development of fruiting bodies appear to go on at the same time and in the same concentration. The first fruiting bodies generally appear at the point of the initial transfer of the fungus. These fruit bodies are surrounded by actively growing hyphae the protoplasm of which is undergoing certain changes preparatory to pycnidium-formation. Klebs (10) states that even in the Ascomycetes and the Imperfecti a cessation of growth and a subsequent lack of food are essential prerequisites for reproduction. But the examples given by him are relatively few, and apply to simpler reproductive bodies; consequently the results obtained with them are to be interpreted from a simpler combination of conditions. The factors which bring about a cessation of mycelial development tend, apparently, on the face of these observations, to check, rather than to stimulate, asexual reproduction in the Sphaeropsidales.

A number of experiments show that in the Sphaeropsidales vegetative growth and asexual reproduction are parallel within wide limits. Let us first consider the complete withdrawal of food from a normally grown mycelium. Regardless of whether the cultures were grown in the standard medium or in the solution sixteen times the usual concentration, a complete withdrawal of food gave rise, in most cases, to fewer fruit bodies than its constant presence. This is because of the fact that a mycelium from which the food is withdrawn gradually ceases its growth, or else grows very slowly. Since the newly forming pycnidia are nourished by the reserve food of the mycelium when the external food supply is exhausted, and since this reserve food supply, no matter how well the mycelium may have been nour-

ished, is not inexhaustible, the quantity of the fruit bodies tends to be limited. This is especially true in case of such forms as *Kellermania yuccagena*, *Stagonospora gigantea*, *Steganosporium acerinum*, etc., which have very large spores rich in protoplasm and in reserve food. When, however, a mycelium, which is well nourished in a nutrient solution of high concentration, is washed and transferred to a weak medium, a large number of organisms exhibit a sharp increase in their reproduction because, in order that the effect of the stimulus may show (as pycnidia), there must be enough food for their development, in addition to the stimulating effect of the sudden removal of food. Even very weak concentrations of food, furnished just when they are needed the most, increase the quantity of fruiting bodies very remarkably, often as much as the higher concentrations. Apparently in such cases only a comparatively slight amount of food is required, and the mycelium derives it with equal ease either from solutions of high or of low concentration. The fact that young pycnidia require a continuous food supply for their best development is proved by the behavior of *Ascochyta nymphaeae*, *Endothia parasitica*, and *Cytospora mendax*. When transferred from the nutrient solution to distilled water, certain parts of the fruit bodies of these organisms are eliminated entirely, and thus the food supply is economized. The first named of these fungi fails to form pycnidial walls on most of its fruit bodies, the spores appearing either in small separate pycnidia or in naked masses. When transferred from distilled water back to food, pycnidial walls and stromata reappear. Stevens and Hall (24) found that when the spores of two species of *Septoria* were plated sparsely, pycnidia were obtained in the cultures, while, if planted densely, the pycnidial walls were eliminated and hyphomycetous spores appeared instead of pycnidia. Taubenhause (25) observed that fruit bodies in *Lasioidiplodia tubericola* and *Diplodia gossypii* may be borne singly or in stromata, or they may be cespitose when grown in artificial cultures. While he does not analyze the causal factors, the quality and the quantity of the food supply may very likely be of great importance in these variations. Similarly, the stromata of *Endothia parasitica* may be eliminated under certain conditions as can be surmised from the description of Shear, Stevens, and Tiller (23) that "small pustules with spore masses occurred," and that these spore masses were "minute, very numerous." According to Anderson (1) the stroma in *Endothia parasitica* does not precede but follows the initial stages in the development of pycnidia. If this statement is true, although Shear, Stevens, and Tiller did not find it to be so in every case, then it becomes easier to conceive how a limited food supply may eliminate such less essential parts as stromata. Another example may be given here to illustrate the same point from a different angle: *Stagonospora collapso*, which normally produces its pycnidia singly, gave rise to a number of well developed stromata when transferred from a weaker solution to a high concentration of food. Here, then, large amounts of available food were necessary for full stromatic development.

If we now examine columns IV and VI of table 5, we shall see that a number of organisms when grown submerged in either the standard or the concentrated nutrient solutions and then transferred to distilled water, gave rise to very few fruit bodies. When, however, after this period of starvation they were transferred to the standard nutrient solution, a sharp increase took place in their fructification. Table 8 illustrates this point very well. Apparently the cultures formed pycnidium-initials when transferred to distilled water, but a lack of food hindered their further development. A total absence of food may be a stimulus, but unless food, even in a very dilute form, is furnished to the mycelium, the effect of the stimulus remains imperfect.

The results tabulated in column II of table 7 show that twelve organisms yielded a hundred percent fruit-body formation when they were transferred from the standard medium to the solution sixteen times the usual concentration. *Ascochyta nymphaeae*, which failed to form a single pycnidium when kept constantly in the high concentration, covered every available spot of the substratum with pycnidia after it was transferred from the low concentration to the high concentration of food. It appears that the protoplasm of the mycelium, after a period of vegetative growth in the standard solution, undergoes certain changes which favor the asexual reproduction more than formation of sterile mycelium; then, upon being transferred to a new condition, these changes persist, and even lead to a richer fruiting. But what about those organisms which remained unaffected, or even showed a decreased reproduction? Of course we can not expect all the organisms to exhibit such finely adjusted balance to the physiological factors as to respond to them with mathematical precision. The quality and the quantity of the nutrient medium used are not, and can not be, just as well suited for one organism as for another, nor can they be capable of maintaining an exactly harmonious balance between growth and pycnidium-formation in all fungi. *Endothia parasitica*, which showed an actual decrease of pycnidia when transferred to the high concentration, gave rise to a very large number of fruit bodies, provided that it was first grown submerged in the high concentration and then was washed and transferred to distilled water, kept there until the fruit bodies began to form, and then transferred directly to the high concentration again. The starving period apparently started a very large number of pycnidium-initials, which matured readily under the influence of the rich food. Thus a period of starvation followed closely by a period of rich feeding induces a maximum fructification.

The carrying-over effect of the mycelium, as well as the ability of a fungus to utilize unfavorable nutrient solutions to best advantage when given the proper environment, can be found well illustrated in table 9. Fungi which failed to fruit in solution B, and made only a poor vegetative growth, formed their fruit bodies in much greater abundance when they were thoroughly washed and transferred to solution A. Certain potential-



ties were acquired by the mycelium as a result of this stimulation and were carried over to solution *A* in the protoplasm of the hyphae. But why did solution *B* inhibit reproduction if it contained such a stimulus for fruiting? It is a well established principle (Klebs, 13) that too heavy concentrations of foods may prove inhibitory to reproduction in many organisms; also the presence of certain unfavorable factors may arrest the beneficial effect of other substances. After these inhibitory substances are washed off from the surface of the hyphae, and those present in the protoplasm are largely neutralized or converted by the assimilation of more favorably balanced nutrient substances, then the stimulus becomes effective. But not all the organisms exhibited the above-described reaction when transferred from solution *B* to solution *A*. Perhaps the influence of solution *B* carried over in the mycelium was too intense for these organisms, and hence could not be overcome by the favorable factors. The positive influences, just as the negative ones, may be carried over in various degrees of intensity, as illustrated in column VI of table 9. When the organisms were transferred from solution *A* to solution *B*, fourteen of them were able to form fruit bodies as against six in the checks. The different potentialities acquired by these cultures during their vegetative growth in the more favorable medium partially or wholly overcame the inhibiting influence of solution *B*. Often the struggle between these two influences lasts long enough for a fair number of pycnidia to appear, but the unfavorable medium, because of its larger quantity and constant supply, eventually overcomes the opposing influences, and the fruiting comes to a halt. In other cases the initial vigor is not strong enough to promote the formation of even a single pycnidium. A different type of reaction which should be observed here is the phenomenal increase in the number of pycnidia of some organisms after a transfer from solution *A* to solution *B*. Apparently the stimulus in this case is carried in solution *A*, and the second solution gives it an opportunity for expression.

#### SUMMARY

1. Twenty organisms belonging to the order Sphaeropsidales were used in these experiments.
2. An especially favorable culture medium was devised; all the twenty organisms produced pycnidia readily on it.
3. A method, which made it possible to wash and completely free the cultures from one solution, and to transfer them to another one as often as it was desired, was developed and used exclusively throughout the work.
4. When light was excluded, two organisms failed to form pycnidia, twelve showed a reduced reproduction, while the remaining six were unaffected.
5. A temperature of 30° C. generally induced a better pycnidium-production in spite of the absence of light, and in the case of one organism, which failed to fruit in the dark and at room temperature, it replaced the

effect of light. A constant temperature of 8° C. inhibited pycnidium-formation in only nine organisms in spite of the fact that light was excluded. In the presence of light, however, all the organisms were able to form pycnidia at a constant temperature of 8° C.

6. A decreased supply of oxygen suppressed fruiting in three organisms, reduced it in eleven, while six remained indifferent.

7. Adsorption was found to be no controlling factor in growth or reproduction so long as an abundant supply of moisture was present.

8. Eight organisms fruited when submerged as well as on the surface; three formed pycnidia but failed to mature the spores, and nine fruited only on the surface of the nutrient solution.

9. Solidity of substratum is a factor in so far as a support is necessary for the mycelium and to bring it up to the air, and possibly to increase food-concentration by exercising a negative adsorption.

10. Generally a higher food-concentration produces more numerous pycnidia. Rich hyphal growth and pycnidium-development are parallel within a very wide range.

11. A sudden complete withdrawal of food from a mycelium grown in a very rich solution is not, in most cases, conducive to better reproduction. If, however, such a mycelium is transferred to a dilute nutrient solution instead of to distilled water, a much better reproduction follows.

12. The sudden increase of food-concentration upon a mycelium grown in a more dilute solution is the condition most favorable to pycnidium-formation.

13. If a mycelium grown in a weak nutrient solution is transferred to a fresh supply of the same solution for five consecutive days, the effect, in many cases, approaches that of the highly concentrated nutrient medium.

14. Osmotic pressure is not a factor in growth and reproduction of these organisms.

15. No evidence of auto-intoxication was seen in any of the cultures.

16. A richly fed mycelium, after a subsequent period of starvation, if transferred to a dilute nutrient solution, gives rise to a great abundance of fruit bodies.

17. The mycelium may acquire certain tendencies which may not manifest themselves unless they are subjected to the action of changed environmental factors.

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## CYTOLOGICAL STUDIES ON GASTERIA

### I. CHROMOSOME SHAPE AND INDIVIDUALITY

WM. RANDOLPH TAYLOR

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The writer has been engaged for some time in a study of the organization and behavior of the chromatin material in the nuclei of *Gasteria*, and the present paper is a report upon the results of observations on the shape of chromosomes in cell divisions in different parts of the plant. The normality of certain features of chromosome shape has been well established in animals, and recent work on plants, especially that of Sakamura (10) has indicated that in these also there were cases of recognizable characters in addition to relative size.

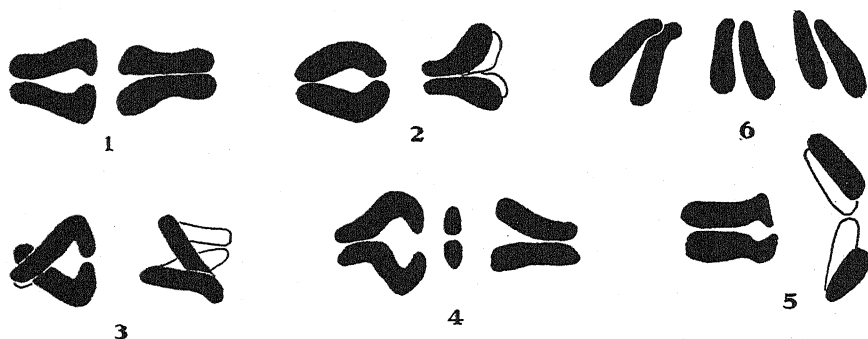
The genus *Gasteria* comprises a considerable number of succulent liliaceous plants native to South Africa, closely related to, and formerly placed in, the genus *Aloë*. Although the species differ evidently in appearance, some of the forms available to the writer in cultivation do not fall sharply into the accepted species, and there is indication of hybridization both in appearance and in pollen sterility. As there does not seem to be any visible difference in the number or the morphology of the chromosomes in the several forms studied, a complete listing of them will not be attempted for the present. Most of the material came from plants fitting best the descriptions of *Gasteria verrucosa* (Mill.) Haw. and *G. Cheilophylla* Baker or intermediates between them.

For the present report the method of paraffin sections was used only for the study of diploid vegetative cells, or for checking up other methods. For the most part the developing microspores were spread upon the slide, fixed, stained, and mounted in position. In this way a very accurate fixation was possible, and furthermore entire nuclei were obtained—an important matter in working out the complete chromosome complement of selected cells. A chrom-osmo-acetic fixing solution gave best results, several modifications being tried. Bouin's picro-formol-acetic with the addition of chromic acid gave usable fixations of tissues, but the results were flashy and suitable only for grosser features of organization. Observations on living cells and on cells stained in aceto-carmin offered no advantages for the present purpose over the fixed smears, but confirmed the more obvious features shown in them.

The drawings illustrating this article have been reproduced to give a magnification of 2000 diameters.

## OBSERVATIONS

Of all tissues of *Gasteria* that were studied, the one in which differences between the chromosomes were least evident was the first (heterotypic) maturation division of the microsporocytes. The three small chromosome pairs, each pyriform and with the apex directed toward the pole, contrast strongly with the four large split pairs. There are no differences to be observed between the members of the first group. On the other hand, when viewed from the side at metaphase it is seen that a certain pair of the second group separates differently from the other three pairs. These always pull apart by their inner tips, even though the outer ends of the pair seem to separate first at times. The odd pair pulls apart in a curved fashion, the ends remaining longest in contact, and the shape of the curve indicates a fiber-

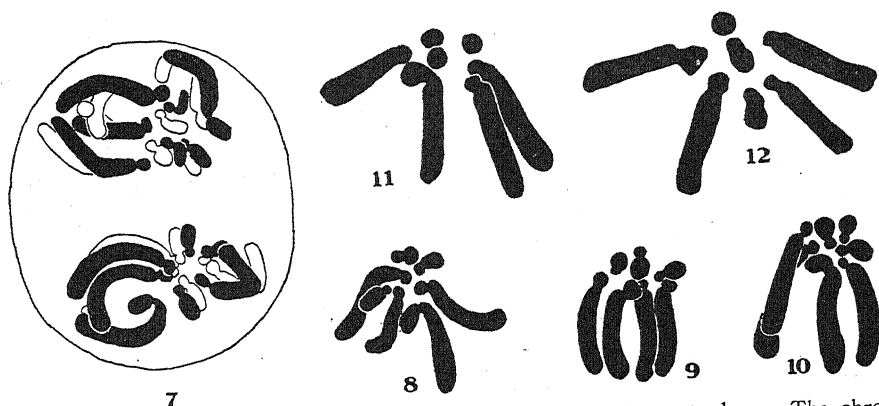


TEXT FIGS. 1-5. First maturation division, microsporocytes, metaphase or very early anaphase. FIG. 6. Same division, late anaphase. Each figure shows two or more homologues from the same complex; the chromosome on the left in each case has the fiber-attachment one third the distance from the inner end. The more distant half of each homologue where visible is shown merely in outline.

attachment about one third of the length distant from the inner end (figs. 1-5). It is frequently to be noticed that another one of the large series moves toward the pole considerably in advance of the rest, so that before the other chromosomes have separated the members of this pair are stretched at full length pointing toward the pole (fig. 5). The writer was not able to assign this precocious character to any definite pair, or to correlate it with any other character. As the chromosomes of the large series approach the spindle poles, the split in each widens and the halves usually separate completely, although remaining near each other. The odd chromosome can still be recognized by its curved tip, but the distinction can be made only when the chromosome is viewed from a favorable angle.

Conditions are better for study during the second (homoeotypic) maturation division. Here the chromosomes are more elongate, and come out of the resting phase by a so much less complicated series of stages that their formation can be more readily followed. The split which separated the

halves of the chromosomes in the first division persists, so that for this second division they appear widely separated in the earliest prophase spireme. At metaphase the corresponding daughter chromosomes arrange



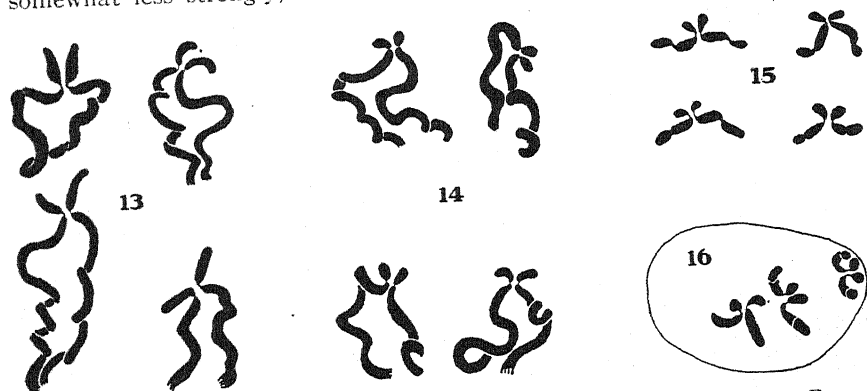
TEXT FIG. 7. Second maturation division, microsporocyte, metaphase. The chromosomes do not pair much more closely than shown. The lower set of chromosomes in each plate are indicated in outline. FIGS. 8-10. Same division, oblique views of single anaphase groups. FIGS. 11, 12. First division in the microspore, oblique views of single anaphase groups.

themselves in a fairly regular plate. It is then evident that the three small chromosomes and three of the large ones in each group have each a rounded protuberance at one end. The odd large chromosome has a very considerable segment separated by a constriction from the main body (fig. 7). In anaphase this chromosome is bent at the constriction (figs. 8-10), and it can be seen that the spindle fiber is attached there. The significance of the smaller constrictions near one end of each of the other six chromosomes is not so obvious, but it was determined with reasonable certainty that the spindle fibers were attached to these not at the tip of the knob, but at its base in the position of the constriction. This agrees with the figures in Sakamura's paper (10), especially with numbers 5 and 6 on Plate 1.

It might be considered that the position of the attachment of the spindle fiber determines the position of the constriction, but this does not seem to be the case. The late prophase stages of the second maturation division of the microsporocyte afford the very best opportunity for study of the position of these constrictions, long before any fiber-attachment has been effected. As the spireme shortens, it becomes evident that at certain points two strands approach each other closely, are there constricted, and beyond the constrictions are produced into divergent segments. The smaller chromosomes show up most strikingly at this stage as more or less "x"-shaped objects (figs. 15, 16). Because of the elongate character of the spireme parts it is not to be expected that one can see in each nucleus all seven of the pairs of strands at the points where they come together, but nuclei showing

three or four are common, and the writer has ascertained that all seven pairs of strands really are so arranged.

The divergent shorter segments of the pairs show characters in line with what one might expect from the form of the chromosomes at metaphase. In one of the larger pairs they are long and somewhat tapering, whereas the other three large pairs have very short oval ones. Special interest attaches to the small chromosomes, where the longer body portion is again, but somewhat less strongly, constricted (fig. 15). At least two in each cell



TEXT FIGS. 13-16. Second maturation division, microsporocyte, prophase. FIG. 13 shows four examples of the long chromosome with the large terminal lobe, and FIG. 14 four of those with the small terminal lobe. FIG. 15 illustrates the second constriction in four of the small chromosomes. FIG. 16 shows three of these small chromosomes in one nucleus, but they are not in position to show the lesser constriction, as they were strongly bent out of the plane of view.

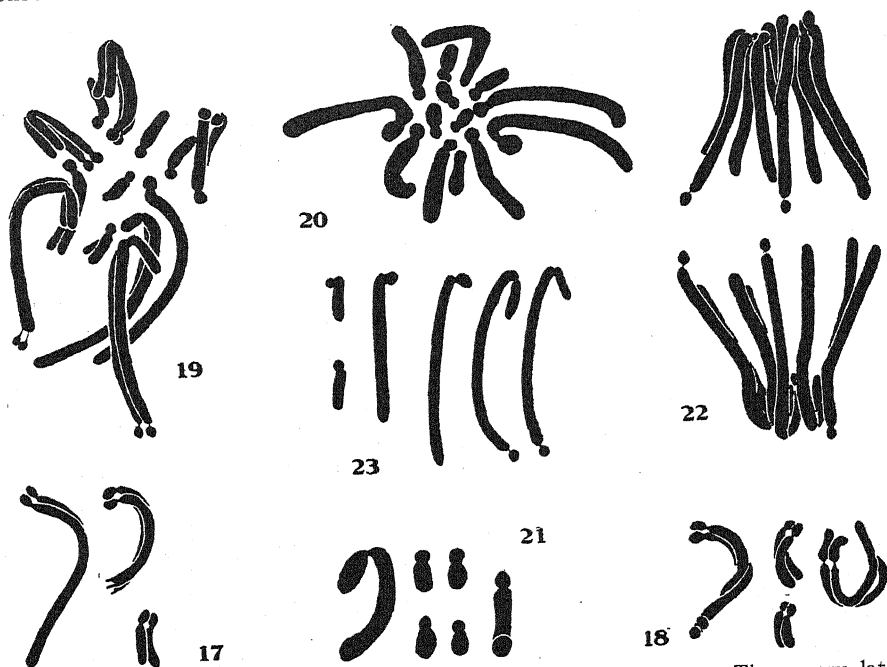
normally show this, but the writer has not succeeded in finding all three of them in a nucleus in the right stage and position to determine if they are all alike. The spindle-fiber attachment is related to the first-described subterminal, not to this more median, constriction. Constrictions of this second type were sought in the large chromosomes but without definite results, partly because their twisted character interferes with a comparison of the sister chromosomes. The positions of fiber-attachment which the writer has described for *Gasteria* seem to be constant for the genus. In some organisms the chromosomes show peculiarities which are constant for individuals in the species, and the constancy of the fiber-attachment is demonstrated by the results of breeding individuals differing in this respect together, where the peculiarities of the parental chromosomes reappear in the hybrid offspring in approximately the ratios to be expected. This has been demonstrated by Carothers (1) for *Circotettix* (Orthoptera).

It is not only during the maturation phases that the individuality of the chromosomes is evident, for the corresponding differences appear during the vegetative divisions also. At the first division in the pollen grains the single distinctive large chromosome is evident with a somewhat truncate



terminal segment, while the other six likewise show their smaller sub-terminal constrictions and lobes (figs. 11, 12).

The vegetative divisions in the sporophytic plant are usually so crowded that it is quite exceptional to get a cell in which all the elements in a complex can be observed. However, when such is found (fig. 20) it shows the expected numbers of two large chromosomes with big lobes, six large and six small ones with the lesser terminal lobes. In favorable prophase also these elements can be identified. The spireme is double in preparation for the metaphase separation, and the constrictions occur on each element of the pair (figs. 17, 18). Anaphases are usually more easy to interpret than metaphases. It is especially easy at anaphase to see the bending of the tip of the chromosomes due to the subterminal character of the fiber-attachment



TEXT FIGS. 17-23. Chromosomes from diploid cells. FIG. 17. Three very late double prophase chromosomes from the ovule wall, after the break-down of the nuclear membrane. FIG. 18. Four chromosomes of the same stage and source. FIG. 19. Partial metaphase from root-tip cell. FIG. 20. Polar view of a complete anaphase group in a cell of the perianth. FIG. 21. Six anaphase chromosomes from the male archesporium. FIG. 22. Lateral view of an anaphase in the root tip. FIG. 23. Individual chromosomes from an anaphase complex in the root tip.

(fig. 23). The long chromosome with the large lobe shows in well fixed root cells an additional character in the form of a very small spherical segment at the free end of the chromosome. This segment merges with the main body whenever the fixation is poor, but in actively dividing cells reached quickly

by the fixing fluid it is brilliantly distinct (figs. 19, 22, 23). The writer has studied divisions in root tips, the perianth, the general ovary tissue, and the anther walls and archesporial tissue, with the same general features appearing in each case. In the archesporium (fig. 21) the chromosomes are shorter than in vegetative cells, where they are very long and usually lie with their constricted tips close together (fig. 22).

#### DISCUSSION

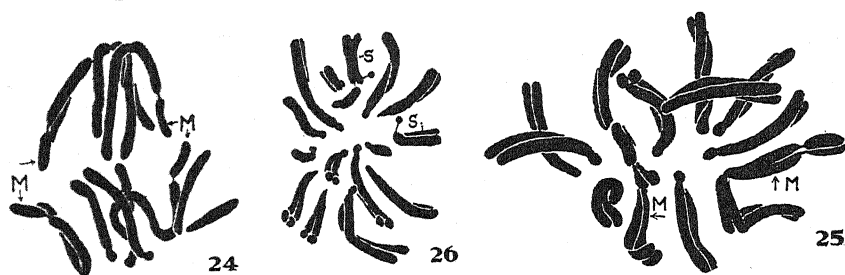
An inspection of *Gasteria* tends to show, then, that certain of the chromosomes possess external features which serve to identify them in any tissue in which they may be observed. The others fall into two groups within which they are indistinguishable by the methods employed, but this does not prove an identical similarity even of morphology, much less of effective organization. Comparable characteristics have been reported for a few other plants, and the number of cases will be readily increased as rapidly as the matter receives attention. For instance, a critical reinvestigation of all cases in which fiber-attachment is median or subterminal will probably show a constriction at the point of attachment, at least in other than the first maturation division. That this is not a mechanical stricture due to the fiber has been shown for *Gasteria* by a study of the prophase stages. Some cases of divergence in reports of chromosome number will doubtless prove to be caused by the interpreting of deeply constricted chromosomes as two or more individuals.

The expectation that plants of close relationship will show chromosomes of similar appearance is justified by many observations, but that this idea can not be safely carried over to the details of constrictions seems indicated by the work of Sakamura (10, p. 20). It is true that in the *Gasterias* which the writer has studied there were no observable differences constant for the several forms. Furthermore, C. Müller (6) gives a drawing of *Aloë Hanburyana* (his fig. 45) which is quite comparable with the related *Gasteria* in having in the diploid complex two large chromosomes with flexed ends, six straight large chromosomes, and six small ones, and, although somewhat crudely drawn, there is nothing to indicate that a more critical study would fail to show agreements in the presence of constrictions themselves. But *Vicia*, a more varied genus than these, shows a considerable diversity of position of the constrictions in the different species. The most studied of these, of course, is *Vicia faba*, where early workers failed to notice the constrictions. Strasburger (12), for instance, does not show them in his figure 195, which deals, to be sure, primarily with spindle-formation. Kemp (3) likewise seems to have missed them here, although working with chloral hydrate material. Fraser and Snell (2), however, saw and figured the constrictions in one limb of the large bent chromosome, but seem to consider that at the bend there is no continuity and that two separate chromosomes exist. Lundegårdh (4) figures many fractures of chromosomes, but true

constrictions seem to be present in his figure 51 and perhaps in his figure 50 also. He observes the tapering of the other chromosomes toward the attachment point, but does not show the terminal swelling. His figure 47 shows the subterminal attachment of the spindle fiber. Sharp (11) finds a transverse segmentation of certain chromosomes, and in anaphase the corresponding daughter chromosomes may be identified by them. Lundegårdh (5) again indicates fractures as present, and also the true constriction in one arm of the large chromosome, but at the bend he draws in the same anaphase in one group the two arms continuous and in the corresponding chromosome in the other group draws them separate. Sakamura in 1915 (9), but more especially in 1920 (10), treats in detail of the conditions in *Vicia faba* in both vegetative mitoses and reduction divisions, figuring both the middle constriction in the large chromosome and that in one limb (his figs. 1, 4). The smaller chromosomes he figures (nos. 3, 5, 6,) with subterminal fiber-attachment, but does not indicate a constriction at this point in normal roots, though he does so in roots treated with benzine vapor (fig. 128), carbon dioxide (fig. 132), and chloral hydrate (figure 64). He reports these agents as increasing the visibility of constrictions recognizable in normal roots, and as showing them in certain definite places when they were not recognizable with his technique in normal roots. He does not think that they actually cause constrictions to arise spontaneously, but merely emphasize an inherent character of the chromosome. Sakamura also studied fresh sections of living roots, getting confirmatory evidence from these. The writer went over the situation in root tips of *Vicia faba*, finding conditions as Sakamura had indicated, but being rather more successful in distinguishing the subterminal constrictions in the smaller chromosomes. A part of an anaphase showing both pairs of the large chromosomes "M" is given in figure 24, and a metaphase in figure 25. In the latter the left-hand "M" chromosome is not in a position to show the constrictions well.

Sakamura studied several other species of *Vicia* in addition to *V. faba*. This work shows the possibility for variation within a single genus. In *V. atropurpurea*, of 14 chromosomes 2 are shorter than the rest and have a constriction somewhat removed from one end. In *V. pseudocracca* the conditions are similar. In *V. cracca*, of 12 chromosomes 2 are relatively long, with one having a constriction near the middle. This is not unlike the conditions in *V. faba*. *V. pseudoorbis* has 12 chromosomes of which 8 are constricted, apparently, from Sakamura's text figure 2 g, four near one end and four near the middle. *V. sativa* appeared to show no constrictions, but *V. unijuga*, which has a somatic number of 24, has 4 similar long chromosomes with constrictions near one end. It is suggested that this is a "tetraploid" species with the large chromosomes doubled in number by the duplication of the entire complex. When tetraploid or even more complex cells result from the action of chloral hydrate, the same duplication of these large chromosomes is effected. In *Lathyrus vernus*, *Lens esculenta*, *Triticum*

*monococcum*, *Zea mais*, and *Fritillaria camtschatensis*, similar conditions are present. Némec (8), Kemp (3), and Sakamura (10) all report extreme median constriction in the chromosomes of *Pisum sativum* under the action of chloral hydrate. In *Butomus umbellatus*, Terby (13) figures cell complexes of 40 chromosomes, of which 10 are quite long, the rest small. The longer chromosomes seem in part to show in the figures constrictions and swollen tips on the end toward the spindle axis (as in Terby's Plate I, figure 12, or Plate 2, figure 15).



TEXT FIGS. 24-25. Mitoses from root tip of *Vicia faba*. In FIG. 24 only part of the anaphase complex is shown, the remainder being present in another section. FIG. 25 shows a polar view of a metaphase; the left-hand "M" chromosome is not in a position to permit a study of its constrictions. FIG. 26. Polar view of a metaphase plate in the root tip of *Galtonia candicans*.

Among other plants perhaps the most notable case is in *Najas* as reported by C. Müller (6, fig. 22) and by Tschernoyarow (14, fig. 1). Here all the chromosomes with the possible exception of one pair have constrictions. Particularly interesting are a pair of comparatively small bodies attached by strands to two of the shorter chromosomes. These, designated "Trabanten" or "satellites," are comparable to similar more minute elements reported as present in *Galtonia* by S. G. Navašín (7, fig. 2, page 379). These latter the writer has found in cells of *Galtonia candicans* root tips, and figure 26 shows them attached to chromosomes marked "S." The subterminal constrictions at the attached ends of the other chromosomes are visible here also at metaphase, but become obscured as the chromosomes pass toward the poles. Probably this is due to imperfect fixation, for Navašín saw them at a late stage.

#### SUMMARY

From the study of the form of *Gasteria* chromosomes, evidence is presented showing a recognizable morphological individuality of certain chromosomes. This is related to the normal constancy in the point of attachment of the spindle fibers, which is demonstrated by the fact that the constrictions where they attach are to be seen in the prophase stages, especially of the second maturation division.

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## A STUDY OF THE FACTORS CONCERNED IN THE REDDENING OF LEAVES OF *DIERVILLA LONICERA*

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### INTRODUCTION

The work on which this paper is based was done at the Biological Station of the University of Michigan at Douglas Lake, Michigan, during the summers of 1919, 1920, 1921, and 1922. Acknowledgment is made to Dr. F. C. Gates, under whose direction the work was done, for very substantial help in planning and setting up apparatus, in suggestions as to methods of work, and in the revision of this paper.

It has been noticed that the leaves of *Diervilla lonicera* Mill. become red under certain circumstances, and an attempt has been made to account for this phenomenon. It is not uncommon to find young leaves of many plants red on first appearing, while the red color of dying leaves is a well known phenomenon. But the color as observed in *Diervilla lonicera* is seen, not in very young or in dying leaves, but in mature, healthy leaves, and therefore is not to be explained in the same way. Since the reddening was more pronounced in plants growing in open, unshaded spots, the natural supposition was that the sunlight was one of the causes of this color change. Also, the fact that the red color was found on plants growing in very sandy and exposed places led to the supposition that the nature of the soil and its moisture-holding capacity, and the water content of the leaves were other factors.

In 1916 experiments were carried on at the Station by Mr. L. Ward McReynolds, who arrived at the conclusion that the reddening is due to extreme sunlight and that the red leaves contain less moisture than the green leaves.

### THE PLANT

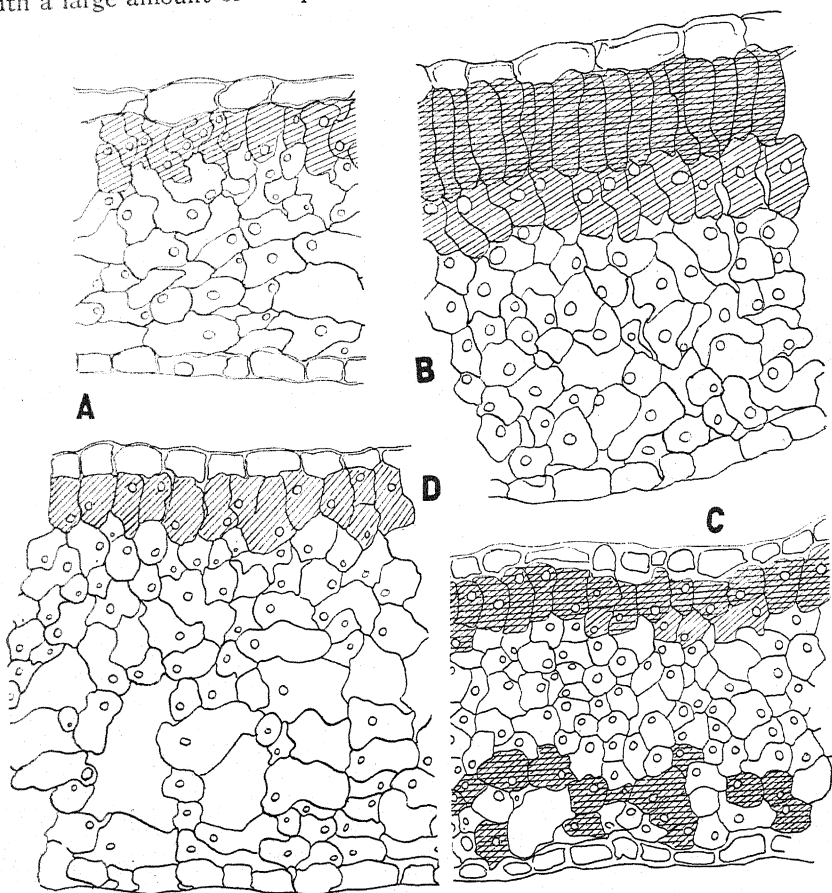
*Diervilla lonicera* Mill., sometimes known as bush honeysuckle, is a low, shrubby plant with oblong-ovate, taper-pointed, opposite, serrate leaves. In Gray's "Manual" and in Britton and Brown's "Illustrated Flora" its habitat is given as "dry and rocky woodlands." In the Douglas Lake region this plant is found in locations varying from exposed sand to boggy woods. *Diervilla* is an abundant low shrub of the aspen association and appears in greatly increased amounts in localities that have been repeatedly burned over. This is especially true where some trees have been left stand-

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ing after ground fires. In May, 1921, such a fire burned over an aspen association of twenty-five years' standing. By July first, *Diervilla* was present in such increased abundance that it formed a dense cover, particularly in that part of the burned area which bordered the untouched woods.

The character of the leaves varies with the kind of habitat. In deep shade the leaves are large, thin, and delicate in texture, while in the sun they are much smaller and thicker. A study of cross sections of typical leaves shows that those grown in the shade have but a single layer of palisade cells, with a large amount of air space in the spongy tissue (text fig. 1, A). In



TEXT FIG. 1. Camera lucida drawings of cross sections of leaves of *Diervilla lonicera* Mill. Cells with crossed lines are red cells, those with only parallel lines are green palisade cells. Nuclei have been shown in the cells to distinguish them from intercellular spaces. A. A typical green leaf showing single layer of palisade cells and large amount of air space. B. A typical red (claret brown) leaf showing greater thickness, double layer of palisade cells, and small amount of air space. C. A red leaf showing red cells in spongy tissue on under side of leaf. D. A leaf, formerly red, which has become green through artificial shading, showing disintegration of double palisade layer.



the red leaves there are commonly two layers of palisade cells and a much smaller amount of air space in the spongy tissue (text fig. 1, *B*). Sometimes, in a very extreme location, red coloring matter will develop in the cells of the spongy tissue on the under side of the leaf (text fig. 1, *C*).

The plant spreads by means of underground rootstocks which lie very near the surface (7.5 cm.). When shade plants are removed to a more extreme location, the aerial parts usually die, but under normal weather conditions the rootstock sends up new shoots.

#### THE PROBLEM

Investigation was carried on in the following manner: color observations were made in direct sunlight, in natural shade, and in artificial shade produced by screens cutting off one half and one third of the sunlight, respectively; a comparison was made of transpiration in red and green leaves, as shown by potometers; the condition of stomates in red and green leaves, whether open or closed, was determined by the use of xylol; a comparison was made between the water content of red and of green leaves, as well as of the soil in which the red and the green plants were growing; the effect of artificial irrigation upon red and green plants in exposed and in shaded positions was observed, and also the effect of irrigation upon red and green plants in three grades of soil in sun and in shade; a comparison was made of color changes in red and in green plants in three grades of soil, in sun and in shade, without irrigation; and a comparison of the hydrogen-ion concentration of the soil in varying locations was made. Standardized Livingston atmometers were used in connection with all experiments.

#### EXPERIMENTATION

##### Color Observations, 1919

In order to conduct comparative observations, locations representing three degrees of exposure were chosen: an open spot among the aspens on the fire line south of "Manville" at the Biological Station, in light, sandy loam, elsewhere designated as "poor soil," will be called station 1; an exposed southern slope on the grade south of Camp Davis, in soil similar to that of station 1, will be called station 2; open sand near station 2 will be called station 2*a*.

At each of these locations two screens, painted green, were set up. The frames were about 44.5 × 41.5 cm., with slats 1.3 cm. wide placed 1.3 cm. and 2.6 cm. apart, cutting off one half and one third of the direct sunlight, respectively. During this year the sides were covered with white cheesecloth to shut off all but the vertical rays. The screens were set horizontally with the slats running north and south. Atmometers were placed under and near each screen, in sun and shade, and were read in connection with each color observation.

The following list gives the complete description of the colors noted:

Color Name	Plate	Color or Hue Number	Tone
Greens: Parrot green.....	VI	31	k
Oil green.....	V	27	k
Calla green.....	V	25	m
Cerro green.....	V	27	m
Cedar green.....	VI	31	m
Reds: Victoria lake.....	I	1	m
Claret brown.....	I	5	i
Nopal red.....	I	3,	i
Pompeian red.....	XIII	3,	k
Madder brown.....	XIII	3,	m
Diamine brown.....	XIII	3,	i
Brazil red.....	I	5	

The characteristic color of shade leaves of *Diervilla* is parrot green.<sup>1</sup> The leaves in the open range from oil green to cedar green. The reddening, usually Pompeian red, occurs first in the midrib. Next the edges turn, at first to madder brown, but later deepening to claret brown. Then a tinge of madder brown appears to overlie the entire leaf; this may deepen to claret brown, the whole surface of the leaf becoming a uniform claret brown. Leaves which are partially shaded by other leaves of the same plant or by such plants as *Pteris aquilina* L., which is abundant in the same locations, are less uniformly red and usually lighter in color. These shades may vary to any of the colors named above, but madder brown and claret brown are by far the most prevalent.

The red leaf usually has two layers of palisade cells. The red color commonly found in the outer layer is due to an anthocyanin which suffuses the entire cell contents. On the other hand, the shade leaves normally have but one layer of palisade cells. Red leaves which have become green through shading lose the arrangement of palisade tissue in two rows and approximate that of typical shade leaves, as shown by figure 1, *D*.

The following paragraphs summarize the color observations taken during 1919.

July 1. At station 1 the shade leaves were parrot green. In the open the majority of the leaves were oil green, tinged with madder brown. Some had the tips and edges of claret brown. At stations 2 and 2a the same color conditions prevailed, except that many leaves were uniformly claret brown on the upper side.

July 8. No change was observed under the screens with the wider space between the slats,<sup>2</sup> but at station 2 some leaves under the narrow screen that had previously been uniformly red were greenish and in others the red had lightened to madder brown.

<sup>1</sup> Ridgway. Color standards and nomenclature. 1912.

<sup>2</sup> Hereafter the screens with 2.6 cm. between the slats will be referred to as the "wide screens," and those with 1.3 cm. between the slats as the "narrow screens."

July 11. A similar change was noticed in the plants under the narrow screen at station 1.

July 15. The plants under the narrow screens at stations 2 and 2a were decidedly greener than the surrounding ones in the open sunlight. The plants under the screens differed only slightly from those outside.

July 22. The same difference was noticed under the screens at station 1.

July 28. Following a heavy rain on July 27, the plants in the open were still red, although there were many leaves green or tinged with red. Both the red and green colors appeared deeper than before the rain, having become cerise green and Victoria lake, respectively. At station 1 the plants under the wide screen were considerably greener than at the last observation and somewhat greener than those outside. Under the narrow screen the plants were all green, with the exception of a small, unhealthy one which later died. At station 2 the plants in the open were either entirely red or dried and dying. The plants under the wide screen were only slightly less red than those outside, but those under the narrow screen were decidedly greener. At station 2a in the open there were no green leaves. They were uniformly claret brown or Victoria lake. Under the wide screen all the leaves were at least overlaid with red, shading to claret brown at the tip or entirely claret brown. Under the narrow screen there were no entirely red leaves; some leaves were green except for red edges and tips.

August 1. At station 1 the plants in the open were much greener. Those under the wide screen were slightly greener than those outside, while those under the narrow screen were decidedly greener. At stations 2 and 2a there was no change in the exposed plants. The plants under the narrow screens were much greener than on July 28. Those under the wide screens had no really green leaves, although there was more green present than in any leaves outside the screens.

August 8. The plants under the narrow screens continued to be decidedly greener than those outside, many leaves being entirely green, while those under the wide screens were nearly as red as those outside.

August 12. At station 1 the only red to be seen in the plants under the narrow screen was in the midribs of some of the leaves. The plants under the wide screen were greener than those outside, but much less so than those under the narrow screen. At stations 2 and 2a the plants under the wide screens were very slightly greener than those outside. Those under the narrow screens were decidedly greener than those outside, but the upper leaves were more or less tinged with madder brown.

August 15. Following a heavy rain on August 13, the plants in the open were greener at all stations. At station 1 the plants under the wide screen were somewhat greener than those outside, but some leaves were overlaid with madder brown and all midribs were red. Under the narrow screen the leaves were all green, with the exception of one shoot which was very lightly overlaid with madder brown. At station 2 the plants under the wide screen

showed no more green than those outside. Those under the narrow screen were decidedly greener. The midribs of the lower leaves were Pompeian red, and a few of the upper leaves were lightly overlaid with madder brown. At station 2a a condition similar to that at station 2 prevailed, except that there was more red present in all cases.

At this date the heavy rains made further observations valueless and the experiment was discontinued.

It was definitely shown by this series of observations that the red color is due at least in part to the action of the sun's rays and varies with the extremity of the location. The plants at station 2a in full sunlight and pure sand showed the most completely red color and the deepest shade of red. It was also shown that, when all the side light and one half of the vertical rays are cut off, leaves already reddened become green again and will then retain that color. Cutting off one third of the vertical rays only slightly controls the reddening.

#### Evaporation, 1919

The records of evaporation for 1919 show a great reduction under screens as compared with that in the open. The average reduction under the screens was approximately that of natural shade. The average rate in cc. per day was: natural shade, 39.6 cc.; wide screens, 43.7 cc.; narrow screens, 40.8 cc.; open sunlight, 79.9 cc. Since the difference between screens cutting off one half and one third of the light is so slight, it is evident that lessened evaporation is not an important factor in itself. Taken in combination with the cutting down of the light, it may be considered one of the secondary factors.

#### Color Observations, 1920

The following paragraphs summarize the color observations taken during 1920.

July 3. Two screens, respectively wide and narrow, as shown in Plate I, figure 3, were set up at station 1 of 1919. They were placed with the slats running north and south and inclined at an angle of the latitude of this region ( $45^{\circ} 15'$ ), on similar unshaded mounds. As in 1919, atmometers were set under and near each screen.

As Ridgway's "Color Standards and Nomenclature" was not available, Klinksieck's "Code de Couleurs"<sup>3</sup> was used in its place. These names have been checked with Ridgway, and the names as found in Ridgway are used.

June 30. At station 1 plants were almost entirely red, from madder brown to claret brown. At station 2 all plants were red, some overlaid with madder brown, others uniform claret brown, while at station 2a the leaves were all claret brown or darker.

June 30. A heavy rain fell, following which all plants became perceptibly greener.

<sup>3</sup> Klinksieck. Code de couleurs. Paris, 1908.

July 1. Plants had become slightly redder again.

July 4. There was little change under the narrow screen. The plants outside were slightly redder than the plants under the narrow screen. The plants under the wide screen were like those outside.

July 6. The leaves of plants outside the narrow screen were decidedly redder than those under it. Some of the leaves under it were all green, some had only the midrib reddened, and others were overlaid with madder brown. There was practically no difference between the plants under the wide screen and those surrounding it. Many leaves were uniformly claret brown, others were overlaid with madder brown.

July 7. Following a heavy rain on July 6, it was cold and cloudy. All plants both under and outside of the screens were somewhat greener.

July 8. The plants under the narrow screen were much greener than those outside. Some leaves were tinged with madder brown. The plants under the wide screen were slightly greener than those outside. Some leaves underneath were green except for the midribs.

July 10. The plants under the narrow screen had reddened somewhat, but were still much greener than those outside. Those under the wide screen showed little difference from those outside.

July 12. The plants under the narrow screen were much greener than those outside, while those under the wide screen were slightly greener. The plants near the narrow screen were more uniformly red than those near the wide screen.

July 13. The plants under the wide screen were greener than on July 12, but less so than those under the narrow screen. In the field at stations 2 and 2a the plants were much redder than at the preceding observations, being uniformly claret brown or darker.

July 14. Following a heavy rain during the night all the plants were perceptibly greener. Those under the screens showed more change than those in the open.

July 17. All the plants under the narrow screen were green except one which was tinged with madder brown. The plants under the wide screen were somewhat greener than those outside, but there were no entirely green leaves.

July 21. Practically the same conditions were observed as on July 17 under the narrow screen. The plants under the wide screen were redder, although still somewhat less red than those outside.

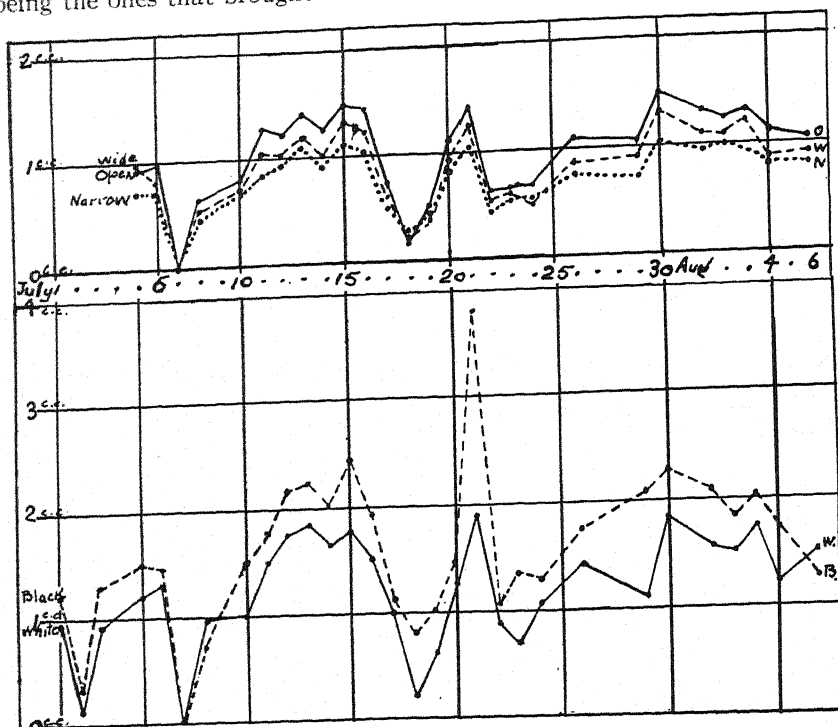
July 24. Following a rain all the plants in the open were a little less red. The plants under the narrow screen were still much greener than those outside; those under the wide screen were slightly so.

July 29. The plants in the open were very red. Those under the narrow screen were the same as at the last observation. Those under the wide screen were somewhat less red than those outside, but there were no green leaves.

August 3. The plants under the narrow screen were still much greener than those outside. Those under the wide screen were becoming redder, there being little difference between them and those outside.

August 7. The conditions were those of August 3. Observations were ended on this date.

This series of observations checked the conclusions reached in 1919 and also showed that cutting off the side light was unnecessary, the vertical rays being the ones that brought about the reddening.



TEXT FIG. 2. Graphs showing difference in evaporation in cc. per hour under wide and narrow screens and in the open (above), and between white and black atmometers at boxes R and G (below), 1920.

The reduction in evaporation under the narrow screen was greater than that under the wide screen, as shown in text figure 2. The average evaporation per day under the narrow screen was 76 percent, and under the wide screen 86 percent, of that outside the screens.

#### Irrigation, 1920

The following paragraphs summarize the effects of irrigation upon color changes. In the following descriptions, station 1 is the same as in previous years, station 3 is an exposed location near the laboratory, and station



4 is a shaded area a short distance southeast of the Biological Station where the soil contains much humus.

June 29, 1920. Plants were removed from the mound at station 1 and from the shade at station 4 to station 3, where they were exposed to extreme conditions of sun and wind for experimentation with irrigation. Boxes  $2 \times 1 \times \frac{2}{3}$  ft. were used, and the earth containing the plants was placed in the boxes with as little disturbance of roots as possible. Each box received a pail of water at the time of transplanting. Atmometers were placed on a level with the plants, a white one at each box and a black one at the box containing the plants from station 1. The box containing the plants from station 1 will be referred to as box *R* (red), and the box containing the plants from station 4 as box *G* (green). The plants in box *G* were of the typical shade color, parrot green. Those in box *R* were oil green, with stems, midribs, and edges of leaves Pompeian red to madder brown. Many leaves were overlaid with madder brown and a few with claret brown. A few of the lower leaves were not reddened.

July 2. Following a rain during the night of June 30 and on July 1, all red plants became somewhat greener and the claret brown in most cases lightened to madder brown. Three irrigators were placed in each box and connected with a reservoir. By this means a large amount of water was furnished to each plant.

July 4. In box *G* the midribs of one plant had become Pompeian red and the edge of one leaf madder brown, while one very young shoot was still parrot green. The plants in box *R* had become decidedly redder, with little green showing. On the evening of the same day it was noticed that many of the leaves in box *G* were lightly overlaid with madder brown and that the madder brown in box *R* had in many cases darkened to claret brown.

July 6. A decided increase in the amount of red in the leaves in box *G* was noticed. Some of the tips of the leaves were claret brown, while all the leaves of the woody shoot were somewhat tinged with madder brown. The herbaceous shoot showed two leaves very slightly reddened. In box *R* all leaves were red, on the whole more so than the plants in the open at station 1.

July 7. Following a heavy rain, all plants were slightly greener.

July 8. The woody shoot in box *G* was claret brown and the herbaceous one was as observed on July 6. Box *R* was very red, most leaves showing no green on the upper side. The plants in box *G* began at this time to lose their leaves gradually. This was due to the extreme conditions, particularly of the wind. The leaves continued to be red on the woody shoot until they dried up, but those of the herbaceous shoot simply dried up.

July 11. Two shoots of box *R* showed a decided increase of green color. These continued to be much greener than those in the field, although the rest of the shoots in the box were very red. These plants seemed to feel no ill effects from the removal, but were able to adjust themselves to the extreme conditions.



July 17. A new crown of *Diervilla* was dug from the woods at station 4 to replace the one which had died. Rain fell during the night, followed by a high wind, which reddened the leaves slightly and dried them. In the course of the next few days most of the leaves dropped off. On July 29, no leaves were left on the plant. Some new leaves appeared on August 15, and remained green to the end of the session.

July 17. The plants in box *R* were still as red as on July 8.

July 19. The under leaves of the plants in box *R* began to show more green.

July 21. The majority of the leaves were still red but some showed only the midrib red; others were merely tinged with red. There were more green leaves than on July 19.

July 26. The plants showed increased red.

July 29. Very few entirely green leaves remained in box *R*. All plants showed increased red.

At the end of the experiment a comparison between the plants in box *R* and those in the location from which they were taken showed the former to be decidedly greener, although the conditions were much more extreme in the new location. The shoots in box *G* were unable to withstand for long the change from the shade to such extreme conditions, but showed a distinct reddening while they lived.

An analysis of these observations shows the following results: red plants taken from an open location to one more exposed but irrigated show a decided decrease in reddening; shade plants taken from woods to an exposed location show a tendency to redden even though irrigated. Evidently the stronger factor is the sun's rays, but the amount of reddening is controlled somewhat by the amount of soil moisture.

The average daily rate of evaporation in 1920, as shown by the atmometers, at station 3 was 21 percent higher than at station 1, and 197 percent higher than at station 4. The black atmometer showed an added increase of 32 percent due to direct insolation (text fig. 2).

A comparison of the soil moisture in the original locations and in the boxes brought the following results:

PERCENTAGE OF MOISTURE IN DRY SOIL

Station 1.....	5.21	Station 4.....	5.56
Box <i>R</i> .....	17.12	Box <i>G</i> .....	39.28

These boxes were allowed to remain undisturbed at station 3 during the winter. Both were found to be in good condition when the session opened in 1921. The leaves in box *G* were green, but of the type found in exposed locations, thicker and smaller than the shade leaves. The leaves in box *R* were red early in June, but after a heavy rain turned somewhat greener and remained so. The boxes were not irrigated in 1921, but each box was given a pail of water early in June and again near the close of a period of extreme

heat in July. At this time the tips of the plants were killed, but new shoots appeared later. The plants in box *G*, on the whole, withstood the heat better than those in box *R* and on August 5 represented a much healthier appearance, due, probably, to the larger amount of humus in box *G*. The leaves of the new tips showed a tendency to redden slightly as they developed.

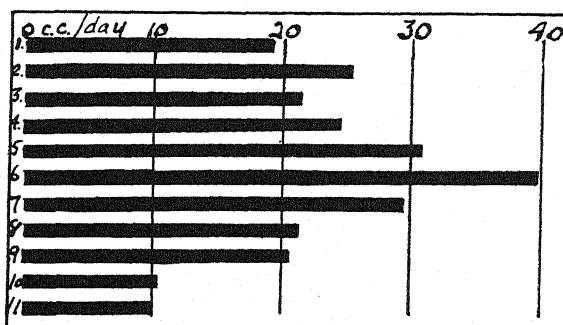
#### Group of Plants Watered without Transplanting, 1920

The following paragraphs summarize the color changes of a group of plants watered where they occurred.

July 22, 1920. A group of plants growing in partial shade and not at all reddened was selected and all shade was removed. One plant in this group was watered regularly.

July 26. It was noted that the plant which had been watered was still green, while those not watered were somewhat reddened.

July 29. The watered plant had reddened slightly, but the leaves of the plants not watered were entirely overlaid with red.



TEXT FIG. 3. Graph giving comparison of average rate of evaporation in cc. per day at varying locations. 1920. 1. 19.1 under narrow screen. 2. 25.3 near narrow screen. 3. 21.4 under wide screen. 4. 24.5 near wide screen. 5. 30.8, white at box *R*. 6. 39.7, black at box *R*. 7. 29.4, white at box *G*. 8. 21.3, sandy bluff near lake, somewhat protected. 9. 20.3, clearing in a protected spot. 10. 10.4, woods where first plants were taken. 11. 9.9, woods where second plants were taken.

August 3. All the plants were much redder than on July 29, but the one which had been watered was decidedly less red than the others.

August 7. The same difference was noted.

The response to the added moisture indicated that the water content of soil is one of the factors controlling the reddening of leaves of *Diervilla lonicera*.

The difference in the average rates of evaporation per day at the various points of observation is shown in text figure 3.

### Comparison of Transpiration of Red and Green Leaves, 1919

This experiment was carried on during one or two days of each week, alternating stations 1 and 2 as locations for the potometers. These were set up as early in the morning as was practicable and readings were taken every hour until about four o'clock, making six or seven readings during the day. Red and green plants were set up in duplicate on each occasion and the area of the leaves was measured. By this means the average rate per hour per 100 sq. cm. was found. Table I gives the weather conditions, rate of evaporation as shown by atmometers in open and shade, and the rates of transpiration of red and green leaves.

TABLE I.

1919	Sky	Wind	Temperature, Degrees F.		Evaporation, cc./hr.		Transpira- tion, cc./hr./100 sq. cm.	
			Open	Shade	Open	Shade	Red	Green
July 4.....	Cloudy	High	85	84	2.27	1.95	1.19	1.77
July 8.....	Overcast	Moderate	76-81	76-79	3.46	2.25	2.01	0.75
July 11.....	Smoky	High	74-80	72-80	2.31	1.80	0.64	0.36
July 15.....	Cloudy	High	58-74	58-73	1.88	0.78	1.25	0.68
July 17.....	Clear	Little	88-93	86-92	2.83	2.09	0.53	0.43
July 22.....	Clear	Moderate	76-82	75-78	3.55	2.08	1.12	0.61
July 29.....	Clear	Moderate	82	80	2.23	1.29	0.85	0.50
Aug. 1.....	Clear	High	74	72	2.45	1.38	0.32	0.40
Aug. 8.....	Clear	High	61-67	57-66	2.80	1.13	0.91	0.31

An interesting correspondence may be observed between the difference in rate of evaporation as shown by atmometers in the open and in the shade, and the difference between the rate of transpiration in red and green plants as shown in table I. While on two occasions the rate was higher in green plants than in red plants, the conclusion seems to be justified that the rate of transpiration in red plants is higher than that of green plants under the same conditions.

### Stomates

Examination of stomates by the xylol method in 1919 showed that, in the majority of cases, on clear days the red leaves had the stomates closed and the green leaves had the stomates open. Leaves which were partly red showed stomates closed in the red portion and open in the green portion. These observations were checked in 1922 and the same results were obtained. This confirms the observations of Stahl,<sup>4</sup> who states:

The cobalt test applied to leaves colored red or yellow in the autumn shows closed stomata. If the leaf is part green and part red, the stomates in the green part are open and those in the red part are closed. Microscopical examination confirms this.

<sup>4</sup> Stahl, E. Einige Versuche über Transpiration und Assimilation. Bot. Zeit. 52: 121. 1894.

### Water Content of Red and Green Leaves and Soil, 1919

Table 2 gives the results of the experimentation expressed as the percentage of water in the moist weight.

TABLE 2.

	July 4	8	11	15	17	22	29	Aug. 1	8
Leaves									
Red.....	66.0%	62.5%	68.7%	62.5%	65.5%	59.0%	62.9%	83.4%	94.5%
Green.....	54.5	70.0	68.5	49.1	63.5	69.0	68.1	70.0	92.5
Soil in the Open									
Surface.....	—	0.0	0.30	0.16	0.0	1.09	7.62*	—	0.02
Root level..	2.39	0.48	0.29	0.56	0.38	0.83	7.43	—	1.65
Soil in the Shade									
Surface.....	0.15	0.14	—	0.42	—	2.46	—	1.41	2.79
Root level..	0.88	0.86	—	0.82	—	0.66	—	3.26	2.78

\* A heavy rain on July 27 accounts for the large amount of water present in the soil on July 29.

In the majority of samples taken, a higher percentage of moisture was found in the soil in which shade or green plants were growing. This was, of course, due to the greater moisture-holding capacity of the humus present in the soil.

The conclusion may be drawn that the small amount of moisture present in the soil of exposed locations is probably one of the factors which helps to bring about reddening of the leaves of *Diervilla*, but, as shown elsewhere in this paper, an addition of moisture to poor or sandy soil will not in itself prevent reddening.

Since in six of the nine samples weighed the red leaves showed higher water content than the green leaves, it is evident that the amount of moisture in the leaves is not a factor to be taken into consideration.

### Color Changes in Red and Green Plants in Three Grades of Soil in Sun and Shade, 1921

Observation of the differences in coloring between plants in the field in locations varying in soil composition suggested an experiment in which a comparison should be made in three grades of soil exposed to the same conditions. Accordingly, a number of both red and green plants were potted to comply with these conditions. The three grades of soil were as follows: good soil, taken from the woods in which green plants grew, containing

much humus; poor soil taken from the location in which the red plants grew, containing much less humus; and pure sand in which no humus was noticeable. A pot of red plants and one of green plants in each grade of soil were placed near the boxes at station 3 and also in the deep shade at station 4. The three pots of red plants at each station were connected with an irrigator, and the three pots of green plants were treated in the same way. Two additional red plants, one in good soil and one in poor soil, were placed at each station but not irrigated.

The heat in 1921 was so extreme that typical reddening in exposed locations did not obtain. As a consequence, the plants at station 3 did not make a normal response to the treatment. The effect of the heat was to disintegrate the chlorophyll instead of causing the appearance of anthocyanin, and the leaves turned brown and died. Even the plants growing in pure sand, which in previous years had been a uniform claret brown, showed only a partial reddening, with many dried and dying leaves. Much of the red seen was the brilliant nopal or Brazil red which shows a dying condition, similar to autumn coloration of other plants.

An examination of the records of color changes during the month of July, 1921, shows the following results:

1. Green plants were unable to adjust themselves to extreme conditions even when irrigated, probably because of unusual weather conditions.
2. Green plants in shade, if in good or poor soil, remained green; in sand they became slightly reddened.
3. Red plants in good soil became greener in extreme locations and in shade lost almost all red.
4. Red plants in poor soil and sand were unable to withstand the extreme conditions; in shade they became but very slightly greener. New shoots arising from old red plants in sand in shade were green. These were of typical shade color, but were small like red leaves.
5. Red plants in good soil without irrigation died in extreme locations but became greener in shade.
6. Red plants in poor soil without irrigation died both in extreme locations and in shade.

These experiments lead one to the general conclusions that:

1. Since green plants in sand even in deep shade tend to redden, the red color is due in part to lack of humus in soil.
2. Since red plants in good soil in shade become much greener, while those in poor soil and sand show only a slight lessening of the red color, again red color depends on the amount of humus in the soil.

These conclusions were supported by observations made in 1922, during which summer there was an unusually large amount of rain, so that all plants thrived even in the most extreme location.

### Hydrogen-ion Concentration

An examination of the soil in which *Diervilla lonicera* was growing, to determine the hydrogen-ion concentration, was made in 1921 and 1922. The determinations were made with standard color solutions made from buffer tablets furnished by the Pyroelectric Company and loaned by Dr. Minna Jewell. The samples were shaken from the roots of plants having red and green leaves respectively, at a depth of about 7.5 centimeters. The results are summarized in table 3.

TABLE 3.

At Root Level	Min. pH	Ave. pH	Max. pH	No. of Determinations
Red.....	5.6	6.0	6.2	7
Green.....	5.6	5.8	6.2	7

### GENERAL DISCUSSION

Observation of plants of *Diervilla* in the field for four years has shown that in more exposed locations the reddening is much more marked than in somewhat protected areas and that in complete shade there is no reddening; that plants growing in sand and soil containing little humus redden more intensely than those which are in richer soil with the same exposure; and that, after a heavy and prolonged rain, the plants become greener. It has also been noticed that in a red plant leaves partly shaded by upper leaves of the same plant are red in the exposed part and green in the shaded part. Leaves which have been used by spiders and insects for nests, in which the tip or side of the leaf has been turned over, are red above and green below.

From these observations it was evident that sunlight, soil content, and water supply were factors to be considered in the investigation of the reddening of this plant.

The experiments with artificial shade showed conclusively that the vertical rays of the sun were the direct cause of the reddening.

Comparison of temperatures under screens which brought about greening of red plants with temperatures in the open and in natural shade frequently showed a higher temperature under the screens. It was therefore judged that the higher temperature in the sunlight as compared with that in the shade was not a determining factor, but that the vertical rays caused a chemical change in the chlorophyl, which could be reversed if the plant was sufficiently shaded.

While the water supply is a factor to be taken into consideration, as shown by the effect of heavy rains on plants in the field, it seems to be a subordinate one. Possibly the lack of sunshine during the rainy weather is as important as the rain itself, as the greening takes place only after prolonged rains. Experiments with irrigation showed a limited control of reddening,



particularly in the plants which were watered without transplanting. Even here the soil content was probably a factor.

A most interesting phenomenon was the behavior in subsequent years of the plants transplanted to boxes in 1920. The plants in box *G* which had been taken from the woods remained green throughout the summers of 1921 and 1922. The soil in this box was covered with a thick layer of leaf mold in addition to the humus already in the soil. In texture and size the leaves resembled the red leaves, but their color was the parrot green typical of the shade plants. The plants in box *R*, taken from the more exposed location in which the soil contained much less humus, reddened characteristically and remained red, varying somewhat in response to heavy rains or dry hot weather.

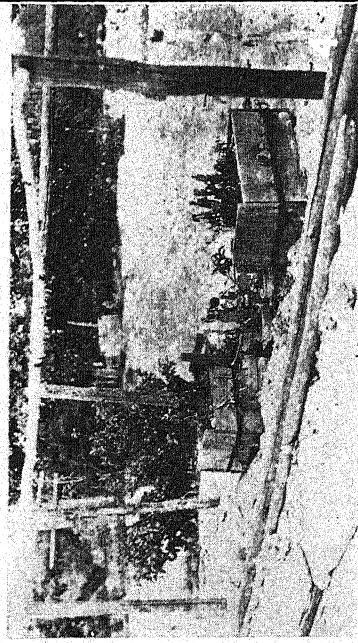
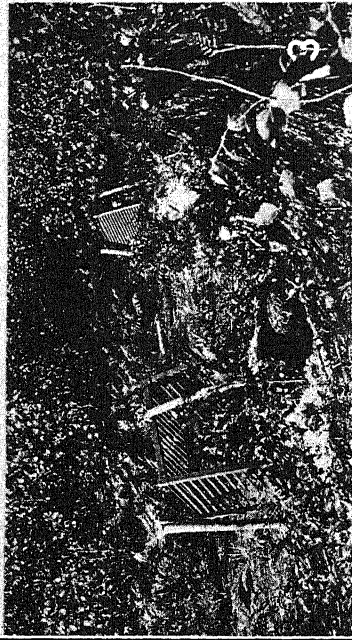
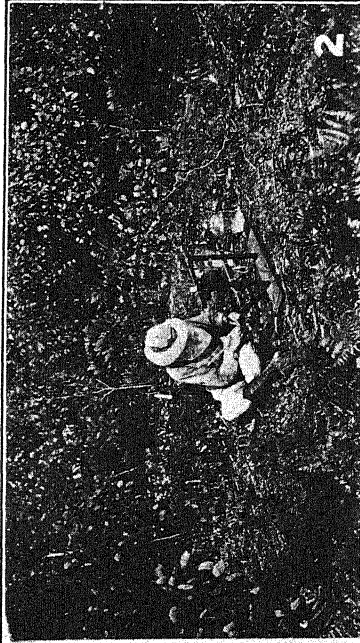
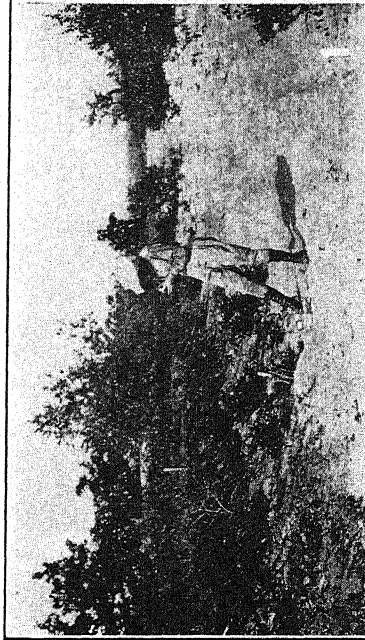
The difference in soil may have influenced the behavior of the plants in at least three ways: greater amount of plant food present; greater moisture-holding capacity of soil rich in humus; larger amount of oxygen in soil containing less humus.

A freeze on the morning of June 26, 1922, which killed the leaves of *Pteris aquilina* L. and of *Rhus glabra* L. and the tops of young aspens, did not injure plants of *Diervilla* growing with them. *Diervilla* also withstands extremes of heat and drought to which the other plants of the association succumb. As it has been observed to be the plants with reddened leaves which are able to resist extremes of heat and cold, it seems reasonable to infer that the anthocyanin present in the leaves functions protectively. Transplanted specimens in the exceptionally hot, dry weather of 1921 were unable to adjust themselves, but under normal conditions the red plants can be moved without appreciable injury or interference with their development.

#### SUMMARY

1. Mature leaves of *Diervilla lonicera* Mill. in the Douglas Lake region redden in sunshine in exposed areas, but remain green in shaded locations.
2. Red plants of *Diervilla lonicera*, if artificially shaded by screens cutting off at least one half of the vertical rays of the sun, became almost entirely green.
3. Transpiration in red plants, as observed by the potometer, was greater than in green plants in seven of the nine cases noted.
4. Stomates of red leaves were closed, those of green leaves were open, as observed by the xylol method.
5. Reduction of evaporation in artificial shade, as shown by standardized Livingston atmometers, approximated that in natural shade.
6. Soil in which red plants were growing contained a lower percentage of moisture than soil in which green plants were growing (average, red plants 1.47 percent; green plants 1.75 percent).
7. Red leaves in six cases out of nine contained a higher percentage of





KEENER: REDDENING OF LEAVES



water than green leaves (average, red leaves 69.4 percent, green leaves 67.2 percent, of moist weight).

8. Red plants in sand, artificially watered, tend to redden even in the shade. Red plants in good soil, artificially watered and exposed to sunlight, show slight reddening.

9. Watering plants by artificial means in a measure controls reddening of leaves.

10. Red plants in poor soil, transplanted to a more exposed location, remained red. Green plants in rich soil, transplanted to a more exposed location, remained green.

11. The hydrogen-ion concentration of soil at the root level of both red and green plants ranges from pH 5.6 to pH 6.2.

12. The factors concerned in reddening are:

- (a) Vertical rays of the sun.
- (b) Kind of soil—whether sand, soil poor in humus, or soil rich in humus.
- (c) Water content of soil.
- (d) Amount of evaporation and transpiration.

#### EXPLANATION OF PLATE I

FIG. 1. Station 2a. Sandy road having most extreme sunshine and leaves showing most intense reddening, 1919.

FIG. 2. Potometer set-up at station 1, 1919.

FIG. 3. Wide and narrow screens at station 1, 1920.

FIG. 4. Station 3, with boxes used in 1920 and pots of red and green plants in three grades of soil, connected with irrigators, 1921.

## THE EFFECT OF HYDROXYL-ION CONCENTRATION ON THE GROWTH OF WALNUT ROOTS<sup>1</sup>

H. S. REED AND A. R. C. HAAS

(Received for publication May 14, 1923)

Repeated observations have shown that Persian walnut trees (*Juglans regia*), as well as other plants, are quite sensitive to alkaline conditions. It is important to know whether the harmful effects observed are to be attributed to the direct effect of the higher concentration of hydroxyl ions upon the plant, or indirectly to the effect of the hydroxyl ion on the nutrient medium. It is well known that many of the essential nutrient ions will be precipitated in the presence of a sufficiently high concentration of hydroxyl ions. Previous investigators have, therefore, found it necessary to use partial nutrient solutions from which calcium, magnesium, and iron have been omitted. In such cases the seed has been depended upon to furnish these missing ions during the early stages of growth.

Hoagland (3) has found that any desired concentration of hydrogen or hydroxyl ions may be obtained by the use of suitable mixtures of the mono-, di-, and tri-basic phosphates together with other nutrient salts. In this way he has obtained solutions with a wide range of hydrogen- and hydroxyl-ion concentration, and has compared the growth of barley seedlings in solutions similar in composition and having like osmotic pressures.

It is a matter of common observation that plants, when grown in water cultures, are able to change the reaction of the medium. In studying the effects of solutions of different hydrogen- or hydroxyl-ion concentration, it is essential that the initial reaction of such solutions be maintained throughout the experiment. This has usually been accomplished by one of the following methods: (a) by renewal of the solution; (b) the use of large volumes of solution; (c) the use of nutrient solutions which have been buffered to resist changes in reaction; (d) a combination of these methods.

The purpose of this work is to determine whether the injurious effects in the case of walnut roots are due to high concentration of hydroxyl ions or to calcium starvation. We have used the method of continuous renewal of culture solution which has since been described by Trelease and Livingston (5), as well as that of employing for each plant a container holding from two to three liters.

The method of growing the seedlings in water culture is shown in figure 1. Carbon-treated distilled water was used whenever distilled water was re-

<sup>1</sup> Paper No. 93, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

quired. Two solutions were chosen from among those used by Hoagland (3), one of which had a pH of 7.37 and the other a pH of 10.85. Neither of these solutions contained iron, magnesium, or calcium.

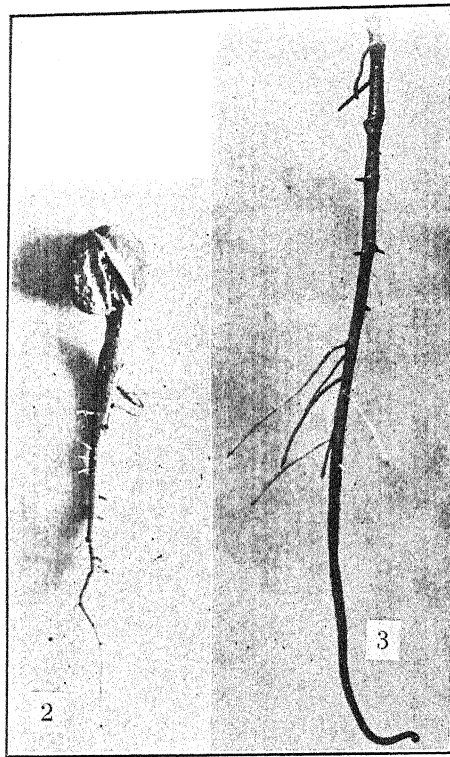


TEXT FIG. 1. A walnut seedling in water culture after several weeks of growth.

When the walnut seedlings had been in either of these solutions for 24 hours or less, injurious effects upon the roots were plainly evident (fig. 2). The root tips became dark-colored, as in cases where discoloration follows the death of a cell with the consequent oxidation of chromogens (4). The injured roots also showed a tendency to become gelatinized even in the short time of these experiments. Frequently a faint reddish coloration was also evident in the dark-colored root tips. Upon removing the alkaline solution after 24 to 36 hours and replacing it with a nutrient solution having a pH of 5.2, the roots began to develop laterals from those portions of the root which had not yet been discolored. Figure 3 shows the type of recovery observed. The white laterals developed after the seedling was transferred to the nutrient solution having a pH of 5.2.

From these and many other observations not specifically reported here,

it appeared that the effects described are due not directly to the high hydroxyl-ion concentration of solutions having a pH value in the neighborhood of pH 9.0, but rather to the fact that this degree of alkalinity removes calcium and other elements from the solution. This idea is strongly supported



TEXT FIG. 2. Walnut seedling grown in a solution lacking calcium. Injurious effects are evident upon the secondary roots. TEXT FIG. 3. Walnut root recovering from calcium starvation. The root was injured by an immersion for 36 hours in a calcium-free solution. The root was then placed in a full nutrient solution containing calcium. The photograph shows the stage of recovery after six days. Lateral roots are being produced from the uninjured portion of the primary root.

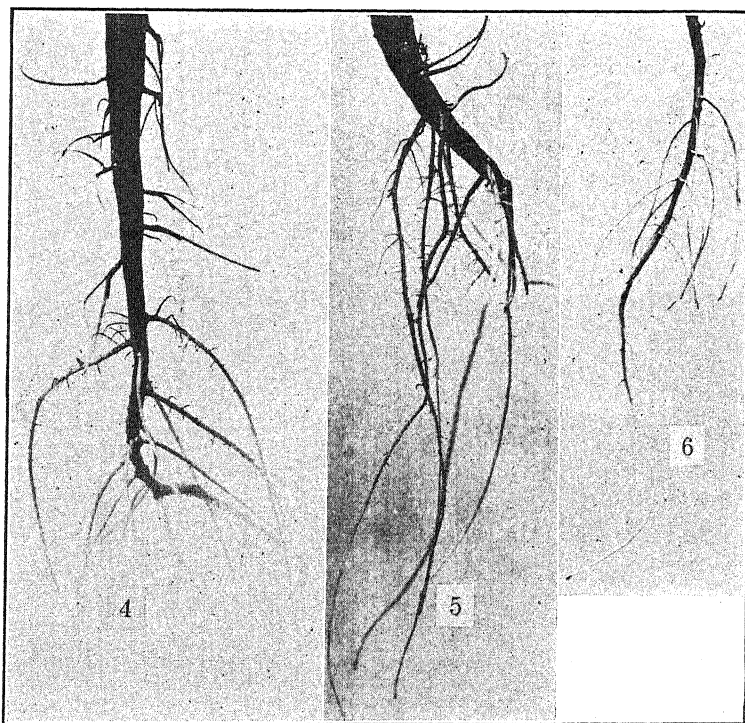
by the fact that equally severe injury resulted when walnut seedlings were grown in Hoagland's nutrient solution (pH 5.2) in which potassium was substituted for calcium. In this latter case there was no precipitation of any salts due to the reaction, and we can ascribe the injurious effects only to calcium starvation.

If, before injury had gone too far, the seedlings were transferred from calcium-free solutions to solutions containing calcium salts, they recovered in the manner already described. Figure 4 shows a good case of recovery following an initial period of calcium starvation. The illustration shows



how freely lateral roots develop from the regions of the primary root which survived calcium starvation.

It was of interest next to ascertain the effect when walnut seedlings were placed in distilled water containing a single calcium salt. It was found that no apparent injury resulted when seedlings were allowed to remain one half hour in calcium hydrate solution with a pH of 9.0 or even somewhat higher.



TEXT FIG. 4. Advanced case of recovery following an initial injury from calcium starvation. TEXT FIG. 5. Walnut roots developed in calcium hydrate solution having an initial alkalinity of pH 9.0 which subsequently was reduced to a value below pH 6. TEXT FIG. 6. Healthy state of the roots after being kept four days in calcium hydrate solution (pH 9.0 or somewhat above). Photograph much reduced.

Seedlings were placed for one half hour in that calcium hydrate solution, and were transferred for the remainder of the day to the previously mentioned calcium-free solutions of different pH values. In every case marked injury was evident after 12 or more hours in the calcium-free solutions.

Other seedlings were placed for 24 hours in calcium hydrate solution, and then were placed for 24 hours in the previously described calcium-free solutions of different pH values. On the following day the process was repeated with the same seedlings. When removed from the calcium hydrate solution,



the seedlings were in excellent condition. They were then transferred to calcium-free solutions. At the end of 24 hours injurious effects became apparent, though they were less severe than when no previous treatment with calcium hydrate solution had been received. The seedlings were replaced in the calcium hydrate solution for 24 hours. They were then placed in the calcium-free solution for 24 hours. At the end of this treatment the injury to the roots became most pronounced, and was unquestionably that of calcium starvation.

Obviously, in a calcium hydrate solution the roots were unable to accumulate sufficient calcium to carry them over a 24-hour period in a solution lacking calcium, although containing other nutrients.

In this connection it may be interesting to cite an analysis of walnut kernels which shows that they are relatively low in calcium (Colby, 1).

Percentage of ash.....	1.13
Soda.....	0.96
Potash.....	12.69
Lime.....	5.57
Magnesia.....	16.60
Phosphoric acid.....	57.83
Sulphuric acid.....	1.31
Silica.....	0.75
Chlorine.....	0.70

Further work is needed to determine whether in the time periods involved there is sufficient passage of ions from the roots into the calcium-free solutions to account for the effects observed. The question is all the more pertinent because Gericke (2) and other investigators have succeeded in growing plants by daily transfers through single-salt solutions which comprised  $\text{KNO}_3$ ,  $\text{MgHPO}_4$ , and  $\text{CaSO}_4$ .

The following experiments were designed to show the effect of strongly alkaline solutions of calcium hydrate.

Walnut seedlings were placed in distilled water containing calcium hydrate (pH 9.0 or somewhat higher), and the solution was changed every 12-24 hours. The pH of the solution was frequently determined, and at no time during the first two days did the pH become lower than 9.0. No laterals had developed, and no doubt the large volume of solution bathing the root prevented any marked change in the pH of the solution. On the third day, however, the lateral rootlets began to make their appearance, and the reaction of the solution was reduced to that of pH 6.0. The solutions were left unchanged thereafter. Obviously, the first two days at pH 9.0 had no evident injurious action upon the subsequent development of the root system. Figure 5 shows the excellent growth attained by the rootlets in this experiment.

In another experiment, seedlings were grown for four days in jars through which fresh medium was continually circulating. In this case the solution

consisted of tap water containing sufficient calcium hydrate to give a reaction of pH 9.0 or somewhat higher. Frequent determinations of the alkalinity of the solution bathing the roots showed that the pH was never below 9.0. At the end of four days the roots had a healthy, white appearance and showed no evidence of injury. No effort was made to establish toxic limits of pH on either side of the neutral point. Figure 6 shows the healthy state of the roots after continuous exposure for four days to calcium hydrate solution of pH 9.0. The darkened tips of the rootlets are the root caps, which are usually dark-colored in any good nutrient solution.

The continuous-flow experiments have been repeated many times without evidence of injury to walnut seedling roots by strong calcium hydrate solutions applied for a period of one week. Growth of these roots appeared to proceed as vigorously for a short time in calcium hydrate solution as in the standard nutrient solution.

When walnut seedlings were placed in distilled water (having a pH between 6 and 7) for one hour, the apical portion of the root showed signs of injury, and after three or more hours they became dark-colored and gelatinous as in cases previously noted when roots were placed in calcium-free solutions of various pH values.

It seems logical to conclude, therefore, that the injury to walnut roots from solutions of high pH values is to be ascribed principally to calcium starvation rather than to the effect of high concentration of hydroxyl ions upon the plant.

No determinations have as yet been made to ascertain the minimal concentrations of calcium necessary to sustain satisfactory growth of walnut seedlings when the supply of calcium is maintained. This is of considerable importance in view of the fact that alkaline soils may be extremely deficient in soluble calcium.

#### SUMMARY

Preliminary experiments have shown that walnut seedlings may be successfully grown for a time in water cultures, and that they are very sensitive to the absence of calcium from the culture solution. Seedlings may be grown for some time in a solution of a single calcium salt, but perish quickly when kept in a culture solution containing all the necessary nutrients except calcium. When the roots are alternately supplied with a calcium hydrate solution and with a calcium-free solution for 24-hour periods, marked injury is evident during the second exposure to the calcium-free solution. Walnut seedlings have been grown successfully for periods of at least a week in solutions of calcium hydrate (pH 9.0 or somewhat higher) which were renewed continuously during the entire period. It seems logical to conclude, therefore, that the injury to walnut roots from solutions of high pH values is to be ascribed principally to calcium starvation rather than to the effect of high concentration of hydroxyl ions upon the plant.

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## SIGNIFICANCE OF THE BEHAVIOR OF SENSITIVE STIGMAS II.

F. C. NEWCOMBE

(Received for publication May 14, 1923)

In a previous paper by the author (9), the experimental study of the closing phenomena of the sensitive stigmas of five species of plants led to the conclusion that the so-called *primary* closing aids in cross-pollination, and the so-called *secondary* closing promotes the germination of pollen on the stigma. The first of these conclusions had been reached by earlier investigators, though not without some dissent, and the second conclusion was without positive support. Within the past two years, there has been opportunity to extend observations and experiments to several other species with sensitive stigmas, and the results of the later study are recorded in the following pages.

### OBSERVATIONS AND EXPERIMENTS

*Spathodea campanulata* Beauv. Growing on the campus of the University of Hawaii, Honolulu, is a group of beautiful young African tulip trees, which bloomed abundantly in August, 1921, showing great racemes of tulip-like, bright red blossoms. This member of the Bignoniaceae has the bilabiate stigma and the didynamous anthers characteristic of that family. The style is negatively geotropic and generally places itself and the stigma between the cluster of stamens and the upper side of the corolla. The flowers open in early morning and remain fresh for eight hours to several days, according to the moisture in the atmosphere. It was a common sight to see all flowers that had opened in the morning wither before sunset of the same day. In the flowers observed, the stigma lobes separated from one another  $90^{\circ}$  to  $180^{\circ}$  as the flowers opened; the anthers opened, in flowers brought to the house, two to three hours later than the opening of the stigma. Self-pollination is thus rendered unlikely both by the proterogynous habit and by the position of the stigma. About one third of all the flowers set seed, though no insects nor birds were observed about the flowers. Close watch for insect or bird visitors was not made. One may well believe that the flowers have visitors in their native habitat at least, for there is such an abundance of honey that it runs out in several drops when the flower is forcibly inverted.

For experimental purposes, 35 flowers, soon to open, were plucked in the evening of different days and taken to the house. Here each flower pedicel was cut through under water, and the flowers stood upright in cups with the pedicels in water. The temperature indoors was  $25^{\circ}$  to  $27^{\circ}$  C., and the air was fairly moist. Such flowers retained their freshness for three

to four days. Pressure by the finger tips or by a folded blade of grass on the inner surfaces of the stigma lobes never caused closing. In some cases there was a slow movement of the lobes, but only through  $10^{\circ}$  to  $15^{\circ}$ . Pollen in abundance was placed on the stigmas of 4 flowers without pressing the stigmas; subsequently none of the stigmas closed though the flowers were watched for three days. On the open stigmas of 5 flowers abundance of pollen was pressed with the tip of the finger. One stigma closed slowly after the lapse of 50 minutes, another after 60 minutes, and a third after 4 hours. The other 2 stigmas did not close. All 5 stigmas kept these positions unchanged until withering. Three open stigmas had pollen applied with the tip of the finger. None closed immediately, but all 3 were closed 20 minutes after pollination and did not subsequently open.

Quite different is the behavior of pollinated stigmas remaining uncovered on the tree. Six open stigmas had pollen pressed on their inner faces by the tip of the finger at 11 o'clock a.m. One closed in five minutes, and the other 5 closed in periods between 5 and 10 minutes. The temperature was  $27^{\circ}$ . This is the shortest period for closing seen in this species. Indoors, the shortest period was 20 minutes. These 6 closed stigmas did not subsequently open.

The stigmas of this species can not be made to close by pressure alone. At least in the scores of trials I have made with flowers, both on the tree and indoors, I have never seen the stigma lobes approach nearer to one another than  $50^{\circ}$  though the stimulation was repeated 10 times within 10 minutes. In the majority of cases of attempted closing of stigmas indoors by gentle pressure of the finger tip or by the blunt tip of a wooden style, no response could be detected. In flowers in warmer air, as on the trees, one could usually detect a movement of the lobes of  $5^{\circ}$  to  $30^{\circ}$ . The shortest latent period observed was 5 seconds.

Wheat flour, when pressed upon the inner faces of the lobes of 3 stigmas by the tip of the finger, caused one stigma to close completely in 20 minutes, one to close to an opening of  $10^{\circ}$ , and the third to an opening of  $20^{\circ}$ . After 3 hours, the one that had closed completely was open  $45^{\circ}$ , the other two were completely open. Two hours later, the first stigma had begun to close again, and it continued its movement till completely closed. The others remained continuously wide open. Three stigmas on which wheat flour was allowed to fall gently so as to cover the whole inner surfaces of the lobes did not show any closing movement though observed for 11 hours.

Sixteen newly opened flowers were tagged on the trees in early morning, and so left undisturbed; in the evening 4 of the stigmas were found closed while the other 12 stigmas were wide open, though several of them were shriveling. This proportion of closed stigmas is about the same as the proportion of fertile to unfertile ovaries, and is probably significant.

Eleven stigmas previously pollinated, in flowers kept in the house, were examined for germination of pollen. Six of these were examined

microscopically six and one half hours after the pollen was placed on the stigmas. Three of the stigmas had been closed six hours and ten minutes, and three had not closed at all. From the closed stigmas, the pollen grains were all swollen and pollen tubes were abundant, from those beginning to protrude to those of a length of ten times the diameter of the pollen grains. The pollen from the open stigmas showed the strongest contrast; there were but a few very short tubes, and the most of the grains had not swollen. Five other stigmas that had received pollen from the tip of the finger 31 hours before were also examined for germination of pollen. Three of these stigmas had been continuously closed for 29 hours or more, and 2 had not been closed at all. Germination had taken place on all 5 stigmas; but from the closed stigmas the pollen tubes were many and long, while they were few and shorter from the open stigmas. Many pollen grains had not germinated at all. All these germination experiments were made indoors; unfortunately, such tests were not made on the flowers in their outdoor environment where the conditions for drying pollen and stigmas were more intense.

Two other members of the Bignoniaceae found in Honolulu were tested merely for sensitiveness of stigma. The shrub *Tecoma stans* Juss. has the bilabiate stigma usual in this family. Probing the flower with a small wooden style, the stigma was found very sensitive, closing in 3 seconds when the temperature was 28°.

The calabash tree, *Crescentia cujete* L., has solitary greenish yellow flowers 6 to 8 cm. long, with very large stigma lobes. I found but a single tree in bloom and could reach but 4 flowers. The stigmas when stimulated by pressure of the finger tip closed slowly, requiring 10 to 15 seconds to effect complete closing. The temperature was 27°.

In May, 1921, *Diplacus glutinosus* Nutt. was found flowering in abundance in the hills to the east of Palo Alto, California. This plant belongs to the Martyniaceae, and the behavior of its stigma has been well described by Lloyd (5). There is only one feature in the reactions of the stigma of this plant that need be added to what is already published. Nine flowers whose stigmas had been given pollen and closed were kept in an open room at an average temperature of 20° for 12 hours after the primary closing. The stigmas had been continuously closed for at least 6 hours after the secondary closing. The cut stems bearing the flowers had been standing in water and all were fresh. They were now placed in a damp chamber at the same temperature as before. After 3 hours, 3 stigmas had opened and 3 others were opening. Fourteen hours after pollination, there was little change. Thirty hours after pollination, 6 were open and 3 closed. Ten hours later, another stigma had opened, while the other two remained closed.

#### DISCUSSION

*Spathodea campanulata* shows, in the behavior of its stigma, an interesting variation from that of all other members of the family Bignoniaceae

so far reported, in that the stigma lobes, though sensitive to pressure, do not close completely on pressure stimulation. The closing mechanism is in a preliminary or in a degenerate stage of development. In favorable outdoor conditions a movement of the stigma lobes may be observed 5 seconds after applying suitable pressure. Similar pressure on the stigma in the cooler and moister air indoors usually brings no noticeable response; or, if there is a movement, the angular distance traversed is less. Stigmas pollinated with the use of pressure while the flowers were on the tree closed in 5 to 10 minutes. Indoors, the closing, under similar treatment, required as a minimum 20 minutes and as a maximum 80 minutes, while some stigmas never closed. If pollen is placed on the stigma without pressure, while the flowers are indoors, there is no subsequent closing. Stigmas once closed, after placing pollen on them with pressure, have never been observed to open.

From the foregoing remarks, it is evident that the first and only closing of the stigma of *Spathodea* is due to pressure and the action of pollen combined; neither alone can cause closing. It may be that the closer contact between pollen and the stigma following application of the pollen by the finger tip is a condition for closing. If so, the closing would probably then be due to causes giving the secondary closing in other species, and not at all to response to pressure. The fact that a stigma was caused to close completely in 20 minutes when wheat flour was pressed on it with the finger tip, while flour alone without pressure never causes closing, lends some probability to the view. That this closing of the stigma waits for the germination of the pollen on the stigma, as believed by Lutz (6) to be true for all species in their secondary closing, evidently does not hold for *Spathodea*; for the closing on the tree, following in 5 to 10 minutes after forcibly applying pollen, is far too early for germination of the pollen. Nor does the quantity of pollen, given without pressure, determine the closing in *Spathodea*, as Lutz found for *Mimulus*; for, without pressure, no amount of pollen will cause the closing of the stigma in *Spathodea*.

The failure of the stigma of *Spathodea* to open after the first closing is the usual behavior of the stigma of *Tecoma radicans* also, though the stigmas of most species, as recorded in literature, open 10 or more minutes after pollination with pressure, to make the secondary closing an hour or more later. But the first closing of the stigma of *Tecoma radicans* is a sensitive response to pressure, requiring but a few seconds for the process; the first and only closing of the stigmas of *Spathodea* is not wholly, at least, a response to pressure, for pressure alone will not close the stigma and it requires 5 to 80 minutes to close when pollen is given with pressure.

Lutz brings some evidence to show that, in natural conditions, it is the abstraction of water from the interior cells of the stigma lobes, coupled with the carrying of some injurious chemical by the penetrating pollen tubes to the interior cells, which causes and maintains the secondary closure. He found that extract of any kind of pollen, even boiled extract,



would cause permanent closing of the stigma of *Mimulus cardinalis*. A few hours after the secondary closing of pollinated stigmas, he found the stigma tissue so disorganized as to be incapable of causing the stigma lobes to open. That such conditions do not obtain for the stigmas of all species with sensitive stigmas is shown by the behavior of *Diplacus glutinosus* as cited in this paper. Nine flowers whose stigmas had been pollinated and had gone through their primary and secondary closing were put into a damp chamber 6 hours after the final closing. Three hours later, 3 stigmas had fully opened and 3 others had started opening. Fourteen hours after pollination there was little change. Thirty hours after pollination, 6 stigmas were fully open, and within the next 10 hours one other stigma opened. Inasmuch as pollen germination takes place 2 to 4 hours after the stigmas close, it is seen that the stigmas are capable of opening in moist air 5 to 30 hours after pollen germination.

While the evidence from all sources seems to indicate that the secondary closing and the continued closure thereafter are immediately due to an increase of permeability of the protoplasm, by which the cell turgor is lost, it is probable that detailed study will show, as is usual in the progress of science, that different species show variations in both causal and responsive phenomena.

As far as my knowledge goes, Lutz was the first author to distinguish a primary and a secondary closing of sensitive stigmas. The primary closing has by several authors been recognized as a means against self-pollination, and I have given evidence in a previous paper to show that the secondary closing promotes, and in some species, in dry air, is a necessary condition for, the germination of the pollen. Study of this subject has now gone far enough for us to recognize the fact that, while most of the species show both primary and secondary closing, there are some species which show primary and no secondary, and there are others which show secondary and no primary closing. *Strobilanthes anisophyllus* as determined by Morren (8), *Strobilanthes isophyllus* as determined by Trelease (10), *Strobilanthes dyerianus* as implied by Goebel, and *Utricularia vulgaris* as shown by Hildebrand (3) have only one functional lip to the stigma, the other being rudimentary. The stigmas of these 3 species are very sensitive to pressure and give the primary closing movement by which the receptive surface is removed from the usual path of a penetrating insect. None of these species can enclose the pollen, for only one stigma lobe is present. The 2-lobed stigma of *Mimulus glabratus* var. *Jamesii* was found by my examination to go through the primary closing, but not the secondary. The flower of this plant has a closed throat. The stigma of *Spathodea campanulata* closes but once, and this closing is the secondary closing of most sensitive stigmas, or, possibly, this stigma combines both closings in one.

The question has been debated by various authors, and especially by Goebel (2), as to the possible advantage to the plant of these two distinct

closures. From the variation in the behavior of the stigmas of different species as cited above, it is evident that it is idle to attempt to bring all species under a single rule of action. In working on this problem, authors have given too little attention to the differing habitats of the species concerned, and to the capability of pollen for self-fertilization. The ecology of the behavior may not be fully demonstrated, as pointed out by Goebel (2), but certain phenomena of behavior have been ascertained.

One fact of behavior is the secondary closing of the stigmas in all species examined except in the four unusual cases mentioned in the last paragraph. And the significance of this behavior is fairly well shown by the relations as summarized in the following narrative: *Catalpa speciosa*<sup>1</sup>, *Tecoma radicans*, *Torenia fournieri*, *Mimulus cardinalis*, and *Diplacus glutinosus* open and remain open after the primary closing if the air about them is very moist; they make the secondary closure and remain closed if the air about them is dry. In the closed flower of *Mimulus glabratus* var. *Jamesii*, in which the air is always moist around the stigma, there is never any secondary closing. In *Spathodea campanulata*, no secondary opening was seen in either moist or dry air, though it should be said that the experiment of using very moist air was not attempted. There can be no doubt that the secondary opening and closing are related to the amount of moisture in the air.

Because Lloyd with *Diplacus glutinosus* and Lutz with *Mimulus cardinalis* found pollen germinating on open stigmas, Goebel (2) draws the rather hasty conclusion that the secondary closing cannot be of much significance in the biology of the plants. But neither Lloyd nor Lutz makes mention of comparing the germination of pollen on the open stigma in moist and in dry air, and Lutz states that the pollen on closed stigmas germinates more quickly than on open stigmas. My own work carries considerably further the evidence for the importance to the plant of the secondary closing of the stigma. Lutz claims that secondary closing cannot aid germination because germination has taken place before secondary closing occurs. This result was not true for the plants I used, many of which made the secondary closing two hours after the secondary opening, and in this period pollen had not germinated on the open stigma, even though the stigma had carried through the primary closing. Forty-eight blossoms of *Catalpa speciosa* showed no germination of pollen on the open stigmas at 22° to 26° C. for a period of 24 hours in moderately moist air, while, in a nearly moisture-saturated chamber, there was abundant germination on the open stigmas in the same time and at the same temperature. Similar results were obtained with the open stigmas of *Torenia fournieri*, *Tecoma radicans*, and *Mimulus cardinalis*. Thus it is shown that a moderately moist atmosphere may prevent the stigmas of several species from closing, and at the same time not provide moisture enough to allow germination. Parallel experiments have shown that few or no seeds were produced in *Torenia fournieri*,

<sup>1</sup> This tree was wrongly given the name *C. bignonioides* in my former paper.

*Tecoma radicans*, and *Mimulus cardinalis* when the stigmas were kept open by moderately moist atmosphere, while abundant seeds were formed if the open stigmas were surrounded by a very moist atmosphere. Details of these results may be found in my publication of two years ago. To this evidence may now be added that given by *Spathodea campanulata*, whose stigmas made no closure at all indoors on the moist Waikiki beach in Honolulu, unless the pollen was applied with pressure. Pollen and pressure together caused closing in 20 minutes. Six and a half hours after pollen was placed on the stigma, 3 stigmas which had closed showed long pollen tubes and plentiful germination, while the 3 which had remained open showed but a few short pollen tubes and the grains generally not swollen. The stigmas of 5 other flowers which had been pollinated in the house 31 hours before, 3 of the 5 having closed early and so continued while the other 2 had remained continuously open, were examined for germination. All showed pollen tubes, but those in the closed stigmas were much the longer and more numerous. This African tulip tree is native both north and south of the equator, bordering the Gulf of Guinea. The region is not well supplied with rain, and one may well believe that the closing of the stigma after pollination is a matter of the greatest biological import in promoting or insuring pollen germination.

#### SUMMARY

The African tulip tree, *Spathodea campanulata* Beauv., offers evidence supporting that of other plants, that the continued closure of the stigma lobes so as to enclose the pollen promotes, and in dry atmosphere is necessary to, the germination of the pollen.

This species is peculiar in that the sensitiveness of the stigma lobes to pressure is not sufficient to cause more than a slight movement, never to cause closing. The complete closing requires both pollen and pressure. The closing does not take place quickly enough to guard against self-pollination; the distant position of the open stigma would seem, however, to make the possibility of self-pollination remote.

The cause of the second closing of the stigmas, as it takes place in most species, must be related to the amount of moisture in the air, since, in the several species so far tested, the closing does not take place if the air is very moist. When the second closing has taken place, a subsequent opening may be induced by placing the plants in very moist air; at least, this result was obtained with the several species tried, and with *Diplacus glutinosus* even after 24 hours of continuous closure.

Besides *Spathodea campanulata* Beauv., *Bignonia stans* Juss. and *Crescentia cujete* L. were found to have stigmas sensitive to pressure, the stigmas of the two latter closing completely with pressure alone.

As listed below, only five families have so far been found to include members with stigmas sensitive to contact. The list given in my former paper

should be revised by crediting the discovery of two species to Koelreuter (4), while others are added from the publications of Treviranus (11), Morren (8), Trelease (10), Miyoshi (7), and Lutz (6). A sixth family, the Lobeliaceae, was reported by Medicus as having species with sensitive stigmas; but sensitiveness could not be demonstrated by Gärtner (1) nor by Goebel (2). Of the five families in which sensitive stigmas have been reported, no species in the Bignoniaceae and the Martyniaceae have been reported with insensitive stigmas. In the three other families, *Torenia exappendiculata* in the Scrophulariaceae was found insensitive by Lutz, *Acanthus mollis* L. in the Acanthaceae by myself, and *Utricularia montana*, *U. uliginosa*, and *Pinguicula* in the Lentibulariaceae by Goebel. All these exceptional cases have the bilabiate stigma characteristic of sensitive stigmas.

In the following list, authors' names are enclosed in parentheses. The Index Kewensis has been employed to eliminate synonyms and to give specific names to two forms for which the authors gave varietal names only.

#### LIST OF PLANTS REPORTED WITH SENSITIVE STIGMAS

Bignoniaceae: *Tecoma radicans* (Koelreuter), *T. grandiflora* (Heckel), *T. stans* Juss. (Newcombe); *Catalpa bignonioides* (Heckel); *C. speciosa* Warder. (Newcombe); *Amphicome arguta* (Heckel); *Incarvillea delavayi* (Burck); *I. olgae* (Lutz); *Spathodea campanulata* Beauv. (Newcombe); *Crescentia cujete* L. (Newcombe).

Scrophulariaceae: *Mimulus luteus* (Henderson), *M. moschatus* (Henderson), *M. [roseus] Lewisii* (Henderson), *M. cardinalis* (Henderson), *M. tillingi* (sp. ?) (Burck), *M. hybridus* (Burck), *M. glabratus* H. B. et K. var. *Jamesii* Torr. et Gray (Newcombe), *M. nepalensis* Benth. (Miyoshi), *M. sessifolius* Maxim. (Miyoshi); *Mazus rugosus* Lour. var. *macrantha* Fr. et Sav. (Miyoshi); *Torenia fournieri* (Burck), *T. [bailloni] flava* (Lutz); *Rehmannia* sp. (Kerner); *Gratiola* sp. (Treviranus); *Digitalis purpurea* L., and *D. purpurea* × *D. lanata* (Newcombe).

Martyniaceae: *Martynia proboscidea* (Koelreuter), *M. lutea* (Heckel), *M. fragrans* (Burck), *M. formosa* (Burck), *M. tricolor* (Lutz); *Diplacus glutinosus* Nutt. (Henderson).

Acanthaceae: *Strobilanthes anisophyllus* (Morren), *S. isophyllus* (Trellease), *S. dyerianus* (Goebel).

Lentibulariaceae: *Utricularia vulgaris* (Hildebrand).

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## SUCCESSION OF FUNGI ON CULTURE MEDIA

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Succession with reference to vascular plants has been studied extensively by the ecologist and by the paleontologist, but very little attention has been given to this same problem with reference to the fungi. Succession, that is, the replacement of one or more species of plants, may require a short or a long period of time. This is usually attributed to changes in the above-ground environmental factors, but comparatively little attention has been given to the study of the underground factors which may be of as great importance as, or even greater importance than, the former.

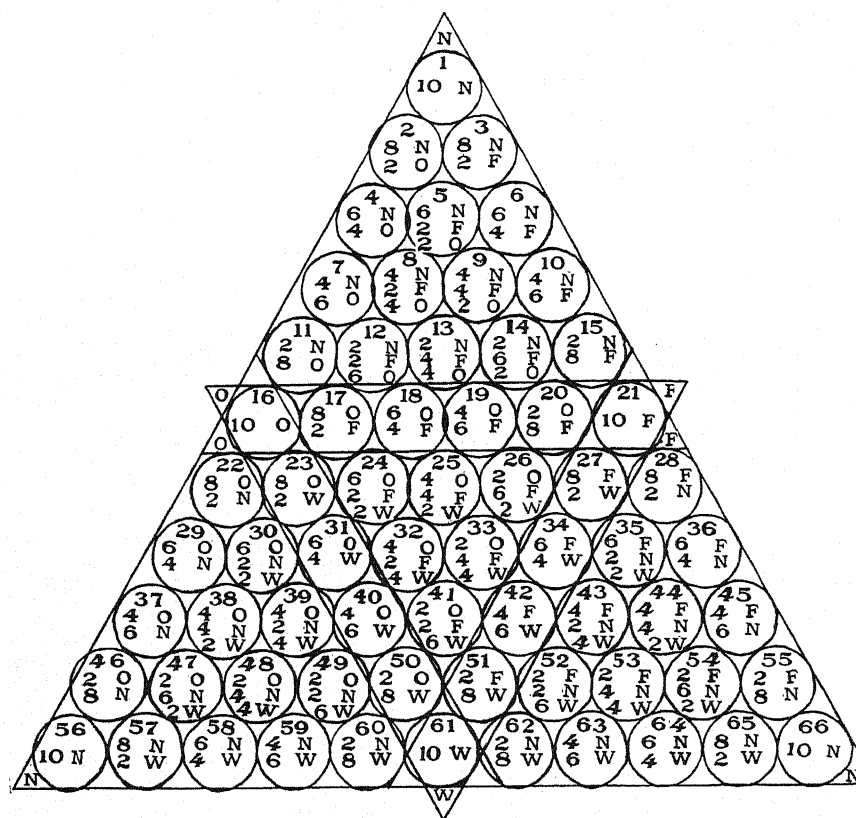
It is well known that species of fungi belonging to widely different groups succeed one another on decaying fruits and vegetables in more or less definite order. Since the period of life of these organisms is comparatively short, there may be a succession of several species of fungi in a very brief period of time. Although the factors influencing succession in the higher plants may be entirely different from those influencing succession in fungi, it is evident that the short period of time involved may result in more definite information in the case of the latter than in the case of the former.

Before entering into a discussion of succession in fungi, the writer wishes to call attention to three fundamental principles which are very generally recognized by ecologists: (1) The functions of the independent plant are dependent on its proper environment. (2) The environment is neither fixed nor constant. Therefore, the individual plants are subject to a somewhat variable environment. (3) In the case of the higher plants the succession or replacement of a species is sometimes very gradual and sometimes very rapid.

In the study of succession of fungi, the displacement is usually rapid and very nearly or quite complete, the aerial environment is probably the same for several successions but the substratum environment may possibly undergo a change; the fungi are usually more mobile than the flowering plants.

The decays of fruits and vegetables are usually begun by fungi that are classed as parasites although they may be partially saprophytic in habit. These parasites are usually followed by recognized saprophytic fungi and bacteria. This is very readily illustrated by the successive growth on apples which decay as result of an attack by *Glomerella rufomaculans*. This first fungus is likely to be followed by a growth of *Rhizopus nigricans* and later by a growth of *Penicillium* sp. No one doubts that the spores of all three of these fungi are present at all periods during the progress in the

decay of the apple, but the question arises as to the factors influencing the order of succession. Since the period of growth for each generation is very short, it is improbable that the environmental agencies above the substratum are of much importance in controlling the succession. Furthermore, this phenomenon of succession has been observed repeatedly in laboratories where the conditions are fairly constant. The most common explanations offered involve (a) character of food, and (b) the possible formation of toxins which kill or check the organism giving rise to them and make the growth of the succeeding organism possible. This study was



TEXT FIG. 1. Diagram showing the arrangement of the beakers in triangles and the medium in each. *N*, new medium, no. II. *O*, old medium, *i.e.*, no. II on which a fungus has been grown. *F*, medium made from fungus removed from medium no. II. *W*, distilled water. Each beaker contained 100 cc. of medium. The number at the top of each circle designates the beaker. The other two numbers indicate the relative amounts of media *N*, *O*, *F*, and *W* in each beaker. Triangle 1 is *N*, *O*, *F*, or 1, 16, 21; triangle 2 is *O*, *N*, *W*, or 16, 56, 61; triangle 3 is *O*, *F*, *W*, or 16, 21, 61; triangle 4 is *F*, *W*, *N*, or 21, 61, 66. The four triangles make up a composite triangle in which 1, 2, 4, 7, 11 duplicate 56, 46, 37, 29, 22; 1, 3, 6, 10, 15 duplicate 66, 55, 45, 36, 28; and 56, 57, 58, 59, 60 duplicate 66, 65, 64, 63, 62.



undertaken in an effort to throw some light on the factors influencing succession.

The experiments were run in three series—*A*, *B*, and *C*—as follows:

#### SERIES *A*

In series *A* the first fungus was grown on Cook's no. II<sup>1</sup> liquid medium and the second in a triangle of beakers in which three constituents were used: new medium, the medium on which the first fungus was grown, and a medium made from the first fungus (fig. 1, triangle *N*, *O*, *F*, or 1, 16, 21). The beakers were of 500-cc. content, about 7 centimeters in diameter and 13 in height. 100 cc. of the medium was used in each. The beakers were covered with two pieces of cheesecloth supporting a thin layer of absorbent cotton. This cover was effective against contaminations and allowed considerable aëration. The experiments of series *A* were as follows:

FIRST FUNGUS	SECOND FUNGUS
<i>Nectria ipomoea</i> .....	<i>Nectria ipomoea</i>
<i>Sclerotinia cinerea</i> .....	<i>Penicillium</i> sp.
<i>Penicillium</i> sp.....	<i>Penicillium</i> sp.
<i>Rhizopus nigricans</i> .....	<i>Rhizopus nigricans</i>
<i>Glomerella rufomaculans</i> .....	<i>Glomerella rufomaculans</i>
<i>Glomerella rufomaculans</i> .....	<i>Cephalothecium roseum</i>

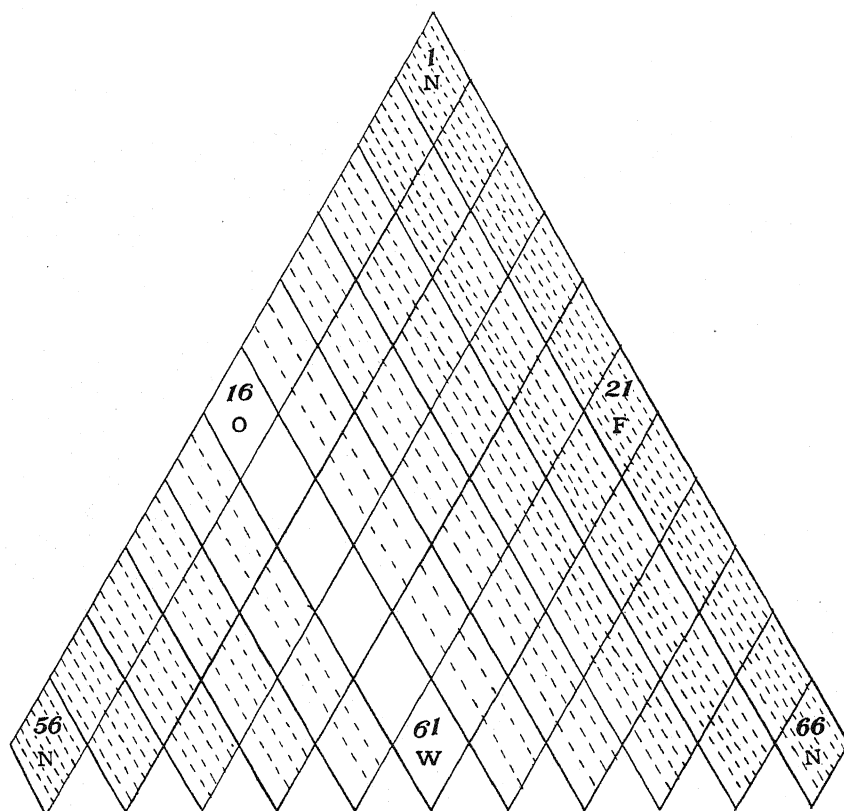
The growth in every case was at its maximum in beaker no. 1, and the same or slightly less in beaker no. 21. There was no growth in beaker no. 16. There was a gradual decrease in the growth from the beakers in the line 1 to 21 across the triangle to beaker 16 (fig. 2). In brief, it is very evident that the fungus can grow very readily in either the normal medium or in a medium made from the first fungus regardless of whether it is the same or a different species, but can not grow in the liquid on which the fungus itself has grown.

#### SERIES *B*

In series *B* the first fungus of each experiment was grown on medium no. II. The second fungus was grown on four triangles of mixtures. The first triangle was made up of new medium, old medium (that is, medium on which the first fungus had been grown), and on a medium made from the first fungus. It was the same as the triangle used in series *A*. The second triangle was made up of old medium, new medium, and distilled water (fig. 1, triangle *O*, *N*, *W*, or 16, 56, 61). The third triangle was made up of a mixture of old medium, medium made from the first fungus, and distilled water (fig. 1, triangle *O*, *F*, *W*, or 16, 21, 61). The fourth triangle was made up of mixtures of medium from the first fungus, distilled water, and new medium (fig. 1, triangle *F*, *W*, *N*, or 21, 61, 66). It was found possible to

<sup>1</sup> Water, 1000 cc.; agar, 15 grams; glucose, 20 grams; peptone, 10 grams; potassium phosphate, 0.25 grams; magnesium sulphate, 0.25 grams.

combine these four triangles into a composite triangle (fig. 1). Each triangle was composed of 21 beakers and the composite triangle was composed of 66 beakers, although there was some duplication. When the second



TEXT FIG. 2. Diagram of the arrangement of the beakers showing the relative growths of the fungi. The growths reached the maximum in the new medium of beakers N. The growth in the diagonal line 1 to 66 was uniform in most cases but occasionally slightly less in 21. The growth decreased in the succeeding diagonal lines to line 16 to 61 in which there was no growth. The growth then increased in the succeeding diagonal lines to beaker 56 in which the growth was maximum.

fungus had completed its growth, the beakers with contents were sterilized in the autoclave and the media were inoculated with the third fungus. Therefore, the records show the abundance of growth of the second and third fungi. The fungi used in this series were as follows:

FIRST FUNGUS	SECOND FUNGUS	THIRD FUNGUS
<i>Glomerella rufomaculans</i> .....	<i>Rhizopus nigricans</i> .....	<i>Penicillium</i> sp.
<i>Physalospora cydoniae</i> .....	<i>Rhizopus nigricans</i> .....	<i>Penicillium</i> sp.
<i>Sclerotinia cinerea</i> .....	<i>Rhizopus nigricans</i> .....	<i>Penicillium</i> sp.

The maximum growth in every case was in the new medium, beakers 1, 56, and 66. The growth on a medium made from the fungus (beaker 21) was approximately the same as, or only slightly less than, that on the new medium. In brief, the growth on the diagonal line from 1 to 66 was practically the same throughout, gradually decreasing on the succeeding lines to line 16 to 61 on which there was no growth. After passing line 16 to 61, the growth gradually increased on each succeeding line to beaker 56, in which the maximum growth was reached. Of course the growths were not quite so uniform as shown in figure 2, but the figure shows the general result of several experiments.

These experiments showed that the fungus in a new medium reaches its maximum growth and that it grows almost or quite as well in a medium made from a fungus. Therefore, it appears that the first fungus furnishes food for a second fungus and does not contain any serious toxic substance. Of course it was not expected that the distilled water would support a growth. The failure of the old medium to support a growth may be due to the fact that the food has already been consumed or to the presence of toxic substances or to both factors.

#### SERIES C

In order to determine this point, a number of species of fungi were grown to the maximum in medium no. II. The fungi were filtered out and the liquid was used in making a medium which was the same as no. II except that the old liquid was used instead of distilled water. This medium was inoculated with several species of fungi, care being taken that each first generation of fungus was followed by the same and by other species. The growths were compared with growths on new no. II medium. In every case the growth in the medium made from a liquid on which a fungus had been grown previously was slightly better than the growth on a new medium. Therefore, it appears that if there is any toxic substance it must have been destroyed or removed by the sterilization. It is more probable that the succession of fungi on a fruit or vegetable or other substance depends on the character of the food. The apple, being much more favorable for the growth of *Glomerella rufomaculans* than for either of the other two, is attacked by that fungus, which, having exhausted the food supply, disintegrates and leaves favorable conditions for a second fungus. The second fungus lives on the food remaining in the apple, which has been made more favorable for its growth, or upon the decaying remains of *Glomerella rufomaculans*, or upon both. These studies very naturally introduce a very important series of biochemical studies which the writer is unable to continue at this time.

These studies were well advanced before the publication of Brown's<sup>2</sup> paper on a somewhat similar line. He finds that some fungi grow better in

<sup>2</sup> Brown, W. Experiments on the growth of fungi on culture media. *Annals Bot.* 37: 105-129. 1923.

the air than in 10 percent carbon dioxid, while others lag for a short time in 10 percent carbon dioxid and then make a growth that surpasses that made in the air. His experiments were made by growing the fungi on agar media in petri dishes, and the comparisons were made by means of linear measurements on the growing surface. He believes that some fungi form two volatile products, carbon dioxid and ammonia, which check the growth of fungi. He says that "these products either diffuse outwards beyond the limits of the growing margins or, as in the case of ammonia, pass into the atmosphere of the culture and from there are absorbed by the cultural medium."

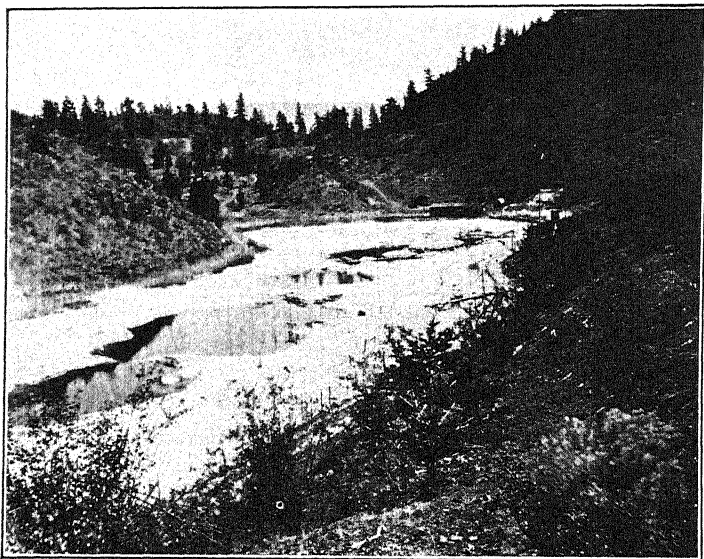
The writer wishes to express his thanks to the New York Botanical Garden for facilities and assistance in carrying on these studies. This work was done while on leave of absence from Rutgers College.

## THE FLORA OF EPSOM LAKE

HAROLD ST. JOHN AND WILBUR DOANE COURTNEY

(Received for publication May 21, 1923)

Epsom Lake is a small lake of nearly pure magnesium sulphate, located near Oroville, Okanogan County, Washington. It has been known for some time that a number of saline lakes occur in this region and in the adjacent parts of British Columbia. This particular one is known locally as "Epsom Lake," "Spotted Lake," "Poison Lake," "Salts Lake," and "Bitter Lake." Its geology has been investigated by Prof. Olaf P. Jenkins<sup>1</sup>, and it was by conferences with him that the writers decided to visit Epsom Lake and to study its flora. With Mr. Charles S. Parker, they spent the day of July 14, 1921, investigating the lake and making a collection of all vascular plants growing in the lake or in its drainage basin. The senior author revisited the lake on July 5, 1923, when he made added observations and further collections. Upon these two collections this report is based.



TEXT FIG. 1. Epsom Lake, showing the solid white salt and, at the center, the dark pools of saturated  $\text{MgSO}_4$  solution.

<sup>1</sup> Jenkins, O. P. Spotted lakes of epsomite in Washington and British Columbia. *Amer. Jour. Sci.* 46: 638-644. 1918.

The authors wish to acknowledge valuable assistance received from Dr. W. J. V. Osterhout of Harvard University, and from Dr. F. L. Pickett and Prof. O. P. Jenkins of the State College of Washington.

This peculiar lake lies at an altitude of 2,000 feet in a depression on the ridge of Kruger Mountain. Here the rock outcrops, which are abundantly exposed, consist of metamorphic rocks, dolomites, and shales. The total drainage area is about half a square mile in extent. Its sides are in most places quite steep, very dry, and nearly bare, supporting only a few scattered trees of *Pinus ponderosa* and *Pseudotsuga taxifolia*. In the low places at the center are several small lakes. All of them are more or less alkaline in character, but one, Epsom Lake, is conspicuously so. It has a queer mottled appearance, the surface being of dazzling solid white salt with a number of dark spots where a dark, slimy liquid occurs. Originally these spots were round and evenly distributed over the surface. Through the changes brought about by the mining operations conducted by the Stewart-Calvert Company, these pools of water are now larger and all connected. It was discovered that the white salt forming the areas between the dark pools consisted of nearly pure epsom salts (99.64 percent).<sup>2</sup> During the World War, when the importations of chemicals from Germany were cut off, the company mentioned mined very considerable quantities of the salt and thus altered the surface of the lake. The salt was found to be so pure that it needed only to be pulverized to be put on the market. The buyers, however, were suspicious of the pulverized salt, so it was dissolved in water, then crystalized out, and sold in this form.

In his report, Jenkins<sup>3</sup> explains that

The dark spots represent shallow pools of brine, immediately beneath which are solid rock-like masses of epsomite. The areas between the dark spots are white because they are dry, and a thin film of an efflorescence of these salts which covers them produces this appearance. Beneath this white film is mud, black, foul, and treacherous, which has been the cause of the miring of cattle in the past. During the rainy season the whole lake is covered with water, and then only a faint appearance of the circles is visible beneath the surface of the fresh water.

Jenkins also explains<sup>4</sup> the method of formation of this lake by drainage from the rocks forming the surrounding basin.

. . . The pyrite and pyrrhotite deposits were oxidized to a depth of several feet from the surface to a mixture of iron oxides, quartz, clay, and tiny crystals of gypsum. Leading from these deposits to the lake were drainage ways, on the surface of which, in places, showed whitish alkali streaks.

<sup>2</sup> A sample of the white salt from the surface of the lake where the plants were growing was submitted to Miss Shirley Holmes, analyst of the Department of Chemistry, State College of Washington. The salt was analyzed three times. The average result was 99.64 percent  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ . Except water, no other substances were present except in traces, there being a minute amount of sodium and of chloride.

<sup>3</sup> Jenkins, O. P., *loc. cit.*, pp. 638, 639.

<sup>4</sup> Jenkins, O. P., *loc. cit.*, p. 643.



These facts suggest the possibility that the sulphates and sulphuric acid, known to form from the oxidation of pyrite and pyrrhotite through the action of meteoric water and air, acted upon the dolomite and other magnesium rocks, forming magnesium sulphate, which is soluble, and calcium sulphate, which is much less soluble. The result was that the magnesium sulphate was carried to the lake in solution. What little calcium sulphate came with it was precipitated first, being less soluble, as a thin layer of gypsum over the sediment already deposited on the bottom of the lake.

Then, briefly, the situation is as follows: a drainage basin of about half a square mile in extent in an area of dolomites and shales, and, in the bottom, a lake of solid, nearly pure  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ .

On the slopes above the lake were found 53 species of vascular plants, in the slimy water of the lake was 1 species, and rooting in the pure white salt were 14 species. The latter formed a noticeable turf near the margin of the white salt, and extended as scattered individuals nearly to the center of the lake. Specimens of all these were dug with a botanical pick. In the case of the perennials with extensive root systems, as the *Distichlis*, it was not possible to dig up the entire root system. However, with the annuals, such as the *Salicornia*, the *Castilleja*, or the *Centaurium*, it can be confidently stated that the whole root system was uncovered, that it was situated in the pure white salt, and that it did not penetrate to any underlying stratum of soil. A sceptical reader may object: How can you be sure that you dug up all of the root system and that the roots did not actually penetrate to some other layer? The writers have stated definitely their observations that the roots did not extend to any different formation; however, this query can be answered from another angle. Nearly half of this remarkable group of plants are annuals. Hence, it must be admitted that, from the germination of their seeds until the plants attained considerable growth, their roots unquestionably must have grown through nearly pure solid  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ .

The preceding paragraph applies to the conditions as observed in 1921. In 1923 they had materially changed. The Epso Products Company, successors to the Stewart Calvert Company, had resumed mining operations. These consisted of pumping to the refining plant large quantities of the mother liquor that formed the dark pools on the lake. As a result, less white salt had been formed on the surface. Digging down a half inch or an inch would expose a layer of black, ill-smelling mud. Hence, in 1923, the majority of the plants growing in the lake, even the annuals, were rooting in the mud below. The relations of the plants to the lake, however, were unchanged. All of the species growing on the lake two years before were still there, but two additional species were found which had escaped detection on the previous visit. All of these plants were still confined to the salt-saturated lake; none of them was found on the rocky slopes around the lake. Conversely, none of the numerous species growing on the surrounding hillsides was found also on the lake.



CATALOGUE OF THE FLORA OF EPSOM LAKE, OROVILLE, OKANOGAN COUNTY,  
WASHINGTON

This includes all plants found in the drainage basin of the lake or on the lake itself.

- Woodsia scopulina* D. C. Eaton  
*Selaginella Wallacei* Hieron.  
*Pinus ponderosa* Dougl.  
*Pseudotsuga taxifolia* (Lamb.) Britton  
*Ruppia maritima* L.  
*Agrostis alba* L.  
*Agrostis alba* L., var. *maritima* (Lam.) G. F. W. Meyer  
*Distichlis spicata* (L.) Greene  
*Puccinellia airoides* (Nutt.) Wats. & Coult.  
*Agropyron caninum* (L.) Beauv., var. *tenerum* (Vasey) Pease & Moore, f. *ciliatum* (Scribn. & Smith) Pease & Moore  
*Hordeum jubatum* L.  
*Scirpus robustus* Pursh, var. *paludosus* (A. Nels.) Fernald  
*Juncus Torreyi* Coville  
*Juncus bufonius* L.  
*Calochortus macrocarpus* Dougl.  
*Salix argophylla* Nutt.  
*Betula microphylla* Bunge  
*Polygonum majus* (Meissn.) Piper  
*Atriplex patula* L., var. *hastata* (L.) Gray  
*Atriplex truncata* (Torr.) Gray  
*Salicornia rubra* A. Nels.  
*Polygonum amphibium* L.  
*Eriogonum heracleoides* Nutt.  
*Silene antirrhina* L.  
*Berberis aquifolium* Pursh  
*Lesquerella Douglasii* Wats.  
*Lepidium apetalum* Willd.  
*Philadelphus Lewisii* Pursh  
*Ribes cereum* Dougl.  
*Heuchera ovalifolia* Nutt.  
*Spiraea corymbosa* Raf.  
*Potentilla Anserina* L., var. *sericea* Hayne  
*Amelanchier Cusickii* Fernald  
*Crataegus Douglasii* Lindl.  
*Holodiscus discolor* (Pursh) Maxim.  
*Prunus melanocarpa* (A. Nels.) Rydb.  
*Rhus Toxicodendron* L.  
*Ceanothus velutinus* Dougl.  
*Opuntia polyacantha* Haw.  
*Centaureum exaltatum* (Griseb.) St. John<sup>5</sup>

<sup>5</sup> *Centaureum exaltatum* (Griseb.) St. John, comb. nov., *Cicendia exaltata* Griseb. in Hook. Fl. Bor.-Am. 2: 69, t. CLVII, A. (1838); *Erythraea Douglasii* Gray, Bot. Cal. 1: 480, (1876); *E. exaltata* (Griseb.) Cov., Contrib. U. S. Nat. Herb. 4: 150 (1893); *Centaureum exaltatum* (Griseb.) W. F. Wight, Contrib. U. S. Nat. Herb. 11: 449 (1906). Prof. M. L. Fernald has pointed out in Rhodora 10: 54, (1908) that *Centaureum* of Hill is the earliest available name for this genus.

*Collomia grandiflora* Dougl.  
*Collomia linearis* Nutt.  
*Gilia pungens* (Torr.) Benth., var. *Hookeri* (Dougl.) Gray  
*Gilia aggregata* (Pursh) Spreng.  
*Teucrium occidentale* Gray  
*Mentha arvensis* L., var. *glabrata* (Benth.) Fernald  
*Castilleja exilis* A. Nels.  
*Orobancha fasciculata* Nutt.  
*Plantago Purshii* R. & S.  
*Specularia perfoliata* (L.) A. DC.  
*Erigeron hispidissimus* (Hook.) Piper  
*Madia exigua* (Smith) Greene  
*Balsamorhiza sagittata* (Pursh) Nutt.  
*Gaillardia aristata* Pursh  
*Achillea lanulosa* Nutt.

The following plants were growing in the water or on the white salt of Epsom Lake:

*Ruppia maritima* L.  
*Agrostis alba* L.  
*Agrostis alba* L., var. *maritima* (Lam.) Meyer  
*Puccinellia airoides* (Nutt.) Wats. & Coult.  
*Distichlis spicata* (L.) Greene  
*Agropyron caninum* (L.) Beauv., var. *tenerum* (Vasey) Pease & Moore, f. *ciliatum* (Scribn. & Smith) Pease & Moore.  
*Hordeum jubatum* L.  
*Scirpus robustus* Pursh, var. *paludosus* (A. Nels.) Fernald  
*Juncus Torreyi* Coville  
*Salicornia rubra* A. Nels.  
*Atriplex patula* L., var. *hastata* (L.) Gray  
*Atriplex truncata* (Torr.) Gray  
*Potentilla Anserina* L., var. *sericea* Hayne  
*Centaureum exaltatum* (Griseb.) St. John  
*Castilleja exilis* A. Nels.

These plants are all limited in their distribution to the salt marshes of the sea coast or to alkaline spots in the interior. In other words, the plants of Epsom Lake itself are essentially halophytes.

This raises the question, what is the similarity in the conditions which accounts for the striking similarity or identity between the flora of Epsom Lake and that of the salt marshes of the sea coast?

The amount of moisture available for plant growth in these two areas cannot be definitely stated, but the amount of precipitation can be compared. At Oroville<sup>6</sup> there was a precipitation of 10.9 inches as an average of the five years between 1917 and 1921, while on the coast of Washington at the salt marshes the amount of rainfall is from two to ten times as much as this. Hence there is no obvious similarity in the amount of moisture at the two places.

There has been a scientific controversy for the last hundred years as to whether it was the chemical nature or the physical quality of the soil that

<sup>6</sup> Climatological data for the U. S. by sections. U. S. Dept. Agr., Bur. of Weather, 1921.

was the important factor in determining the occurrence of plants limited to a particular soil. The most conspicuous example of plants limited in this way is the halophytes, which are so obviously restricted to saline soils that there has been very little question but that the salts present are the determining factors.<sup>7</sup> The common interpretation has been that NaCl is the important factor. With this in mind, a comparison of the chemical composition of sea water with that of Epsom Lake is well worth while.

An average of the analyses of sea water (made by W. Dittmar from seventy-seven samples collected by the "Challenger" Expedition in various areas and at all depths) is as follows:<sup>8</sup>

Sodium chlorid,	27.213 parts per 1000
Magnesium chlorid,	3.807 " " "
Magnesium sulphate,	1.658 " " "
Calcium sulphate,	1.260 " " "
Potassium sulphate,	.863 " " "
Calcium carbonate,	.123 " " "
Magnesium bromide,	.076 " " "

The liquid of Epsom Lake contains practically nothing but  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in a concentrated solution, so concentrated, in fact, that even the difference between the temperatures of day and night will cause the salt to be crystallized out and exert enough force to split, from end to end, a metal pipe containing the liquid.<sup>9</sup> The white incrustation around the border of the lake and on the surface of the solid areas is magnesium sulphate which has lost a part of its water of crystallization. So magnesium sulphate occurs in both; in sea water in 1.658 parts per thousand, and in Epsom Lake as pure crystals or as a saturated solution.

Magnesium salts are very toxic to ordinary plant growth, much more so than the salts of sodium or calcium; however, any of these, except  $\text{Na}_2\text{CO}_3$  and NaCl, are stimulating to plant growth when in dilute solutions.<sup>10</sup> In working with different wheat varieties, Harter<sup>11</sup> found that magnesium sulphate was ten times as toxic as sodium chlorid, as verified by the following results:

Salts	Parts of Normal Solution
Magnesium sulphate,	0.00736 toxic limit
Magnesium chlorid,	0.00931 " "
Sodium carbonate,	0.0109 " "
Sodium bicarbonate,	0.026 " "
Sodium sulphate,	0.0432 " "
Sodium chlorid,	0.0542 " "

<sup>7</sup> St. John, H. A botanical exploration of the north shore of the Gulf of St. Lawrence, etc. Victoria Memorial Museum Mem. 126: 29. 1922.

<sup>8</sup> Murray, J. The ocean, pp. 48, 49. No date.

<sup>9</sup> Jenkins, O. P., *loc. cit.*, p. 644; also testimony of operators at Stewart-Calvert plant.

<sup>10</sup> Harter, L. L. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 79: 24. 1905.

<sup>11</sup> Harter, L. L., *loc. cit.*, p. 41.

In 1907 Miss Burlingham published<sup>12</sup> the results of her experiments on the toxicity of  $MgSO_4$  on seedlings, using young plants of Abutilon, pea, and corn. She concluded that

Magnesium sulphate in solutions of greater concentration than  $m$  8192 has a toxic action on most seedlings, the degree of toxicity varying with the type of seedling and with the conditions.

Similar results were again obtained by Harter and Kearney, who experimented upon the toxicity of the different salts upon different plants:<sup>13</sup>

Plant	Most Toxic Salt	Least Toxic Salt
<i>Lupinus albus</i> .....	$MgSO_4$	NaCl
<i>Medicago sativa</i> .....	$MgSO_4$	$NaHCO_3$
<i>Triticum vulgare</i> .....	$MgSO_4$	NaCl
<i>Zea mays</i> .....	$Na_2CO_3$	$MgSO_4$
<i>Andropogon sorghum</i> .....	$MgCl_2$	NaCl
<i>Avena sativa</i> .....	$MgSO_4$	NaCl
<i>Gossypium barbadense</i> .....	$MgSO_4$	$NaHCO_3$
<i>Beta vulgaris</i> .....	$MgSO_4$	NaCl

From these results it is very evident that magnesium salts are very toxic, that sodium salts are less toxic, and that not all plants are affected alike by the several toxic salts. The last point is stressed by the case of maize—for which sodium carbonate was the most toxic and magnesium sulphate the least toxic.

The results do not necessarily indicate the comparative toxicity of the elements, that is, of the metals themselves, and hence may not be true from the point of view of the chemist. However, they do indicate the toxic nature of the several salts to the plants, and from our point of view give just the data desired.

The work with pure-salt solutions has ordinarily little to do with conditions in nature, because salt marshes, alkali lakes, etc., usually contain a mixture of salts. Experimenting from this angle, Harter and Kearney found that the toxicity of either sodium or magnesium salts was greatly lowered by the addition of some calcium salt, but this again varies with the plant used. More light is thrown on the subject by Osterhout,<sup>14</sup> who concludes that:

1. Plants die much sooner in pure sodium chloride solution (isotonic with sea water) than in distilled water. Addition of  $CaCl_2$  causes them to live nearly as long as in distilled water. When  $MgCl_2$  and  $MgSO_4$  were added they lived practically as long as in sea water.
2. A mixture of solutions which are individually poisonous produces a medium in which the plants live indefinitely.

<sup>12</sup> Burlingham, Gertrude S. A study of the influence of magnesium sulphate on the growth of seedlings. Jour. Amer. Chem. Soc. 29: 1110. 1907.

<sup>13</sup> Harter, L. L., and Kearney, T. H. U. S. Dept. Agr. Bur. Pl. Ind. Bull. 113: 13. 1917.

<sup>14</sup> Osterhout, W. J. V. Bot. Gaz. 42: 127-134. 1906. See also Miyake, K. Influence of the salts common in alkali soils upon the growth of rice plant. Bot. Mag. Tokyo 27: 203. 1913.

From the work of these investigators it is evident that  $\text{MgSO}_4$  is the most toxic of the common salts for most plants. If the salts of certain other metals are present in the proper proportions the toxicity of each is reduced, so that the mixture acts as a balanced solution which is nourishing rather than toxic to plant life.

The following conclusions are drawn from the above discussion:

1. The plants found in Epsom Lake and growing on the nearly pure magnesium sulphate surrounding it are halophytes.
2. The flora of Epsom Lake is identical with, or similar to, that of salt marshes.
3. The only common substance in these two areas is  $\text{MgSO}_4$ .
4.  $\text{MgSO}_4$  is very toxic to plants in general.
5.  $\text{MgSO}_4$  is probably the factor that limits plant growth in both regions, either by killing all plants not tolerant to it, or by its high concentration causing an increased osmotic pressure and thus reducing the amount of available water.

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STUDIES IN WOOD DECAY IV. THE EFFECT OF SODIUM  
CARBONATE, BICARBONATE, SULPHATE, AND CHLORID  
ON THE RATE OF DECAY OF DOUGLAS FIR SAWDUST  
INDUCED BY *LENZITES SAEPIARIA* FR. WITH  
SPECIAL REFERENCE TO THE EFFECT OF ALKA-  
LINE SOILS ON THE RATE OF DECAY OF  
WOOD IN CONTACT WITH THEM

HENRY SCHMITZ

(Received for publication May 22, 1923)

INTRODUCTION

It is still an open question whether or not wood placed in contact with alkaline soils has a longer or a shorter life than wood placed in contact with ordinary soils. Since there are enormous areas throughout the West in which the soil is more or less alkaline, and since relatively large amounts of wood in the form of railroad ties, fence posts, telegraph posts, wood pipe, flumes, etc., are continually in contact with such soil, the question is one of considerable importance.

The action of alkaline soils upon wood may be either direct or indirect. By a direct effect is meant the chemical action of the salts composing the soil "alkali" upon the wood. The only salt found in alkaline soils that need be considered in this connection is sodium carbonate or sodium bicarbonate. Sodium chlorid and sodium sulphate, the other important component salts of soil "alkali," have no important chemical effect upon wood substance or structure. The action of any strong base upon wood is well known, but whether or not the concentration of sodium carbonate or bicarbonate ever becomes great enough in an alkaline soil to injure chemically wood in contact with it is more or less a matter of conjecture, although there seems to be *a priori* some evidence in favor of the affirmative side of this question. It is obvious that, if wood that is saturated with the soil solution of any alkaline soil containing sodium carbonate or sodium bicarbonate in considerable amounts should gradually dry out, the concentration of the salt would gradually increase with the evaporation of the solvent until the solution became quite concentrated. It is conceivable that a constant repetition of this process may have an ultimate effect on the wood. It is not, however, the purpose of this paper to consider the direct effect of alkaline soils upon wood placed in contact with them.

The present paper considers only the indirect effect of the salts making the "alkali" of alkaline soils upon wood. By an indirect effect is meant

the effect of these salts on the rate of decay induced by wood-destroying fungi.

It is also entirely possible that the indirect effect may be correlated with a direct effect, that is, the sodium carbonate or sodium bicarbonate may cause partial decomposition of the cellulose and lignin and thus make them more available to the wood-destroying fungi concerned with the decay.

Dealing more particularly with the effect of these salts on the fungi causing wood-decay, it may be that their presence in small amounts causes a stimulation in growth. The stimulating effect of even very toxic substances when present in minute amounts has been demonstrated, not only in connection with the growth of certain higher plants but also in connection with the growth of certain fungi.<sup>1</sup>

In general, alkali soils contain sodium carbonate, sodium bicarbonate, or both, sodium chlorid, and sodium sulphate in varying proportions, although any of the above-named salts may not be present in excessive amounts in certain alkali soils. Thus it is necessary to consider not only the effect of any one of these salts on the rate of wood-decay induced by fungi, but also the effect of any one of these in the presence or absence of the other two. It may, of course, be entirely possible and indeed highly probable that an antagonistic action exists between the anions of these different sodium salts, that is, between the carbonate, chlorid, and sulphate ions. Quite recently Raber<sup>2</sup> has shown the antagonistic action between the anions of sodium acetate and sodium sulphate on *Laminaria agardhii*.

Early workers in the field, particularly Miss Moore<sup>3</sup> and later Lipman,<sup>4</sup> demonstrated beyond reasonable doubt the antagonistic action between certain anions. The work of Lipman is particularly interesting in this connection. Although all the limiting factors were undoubtedly not taken into consideration in this work, and although the results obtained are probably relative rather than absolute, yet the antagonistic action of the anions of sodium carbonate, sodium sulphate, and sodium chlorid on the ammonifying and nitrifying bacteria is clearly demonstrated.

The present work was undertaken in order to throw further light, if possible, on the question of the effect of alkaline soils upon the rate of decay of wood placed in contact with them.

#### METHODS

A fine sawdust of Douglas fir (*Pseudotsuga taxifolia*) was prepared. The sawdust was sifted through a sieve having a two-millimeter mesh in order

<sup>1</sup> Richards, H. M. Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. 30 : 665-688. 1897.

<sup>2</sup> Raber, O. The antagonistic action of anions. Jour. Gen. Physiol. 2 : 541-544. 1920.

<sup>3</sup> Moore, Anne. On the power of Na<sub>2</sub>SO<sub>4</sub> to neutralize the ill effects of NaCl. Amer. Jour. Physiol. 7 : 315-319. 1902.

<sup>4</sup> Lipman, C. B. Antagonism between anions as affecting ammonification in soils. Centralbl. Bakt. II, 36 : 382-394. 1913.



that it might be uniform in the various culture flasks. Five grams of the air-dried material were placed in 125-cc. Pyrex extraction flasks of known weight. The flasks and contents were then dried to constant weight in an electric oven at a temperature of 102° C. and weighed to the second place. The difference between the two weighings indicates the actual amount of dry sawdust in each flask. By following this method of procedure it was found that there was a little variation in the oven-dry weights of the sawdust in each flask, but in no case was the variation great enough to make any appreciable difference.

Normal solutions of sodium carbonate, sodium bicarbonate, sodium chlorid, and sodium sulphate were prepared in the usual manner. Desired amounts of these solutions were pipetted into a 50-cc. graduate cylinder, after which distilled water was added in sufficient quantity to make 50 cc. of solution. Twenty-five cc. of this solution were subsequently added to each of two of the above-mentioned culture flasks. Thus duplicate cultures were prepared for each concentration. It was felt that by using this method the salt would be more evenly distributed throughout the sawdust and that conditions in the duplicate flasks would be more nearly identical. The culture flasks were then plugged with cotton and sterilized for twenty minutes at ten pounds of pressure. After cooling, the culture flasks were inoculated from plate cultures of *Lenzites saeppiaria* in the usual manner.

The entire series was incubated at 28° C. for 120 days. At the end of the incubation period the plugs were removed from the flasks and the flasks and contents were again dried to constant weight at 102° C. and weighed.

The difference between the original weight of the flasks and their contents and the weight of the same after the incubation period indicates the amount of decay which had taken place in each of the respective culture flasks. The weight of the various salts added to each individual flask must, of course, be added to the loss indicated by the two weighings. From this sum must be subtracted the loss in weight as shown by the uninoculated control flasks. This loss in weight, which amounted to only 0.3 percent, is no doubt due to the fact that the more volatile resinous products present in the sawdust are driven off during the process of sterilization. In all the tables which follow these computations have been taken into consideration, and in all cases the results given are the average of duplicate cultures.

There is a discordant feature in the methods here followed to which attention should be called. It consists in the addition of the salts to the sawdust prior to sterilization. The relatively high temperatures prevailing during this process may produce an effect on the wood which is not duplicated under actual conditions when wood is placed in contact with alkali soils. This discrepancy, however, cannot be very easily eliminated, since it would involve the addition of the salt solutions to previously sterilized sawdust under aseptic conditions. Although this can be done, it would add

greatly to the labor involved in setting up a culture series as here employed. The writer hopes to duplicate this work at some future time modified as suggested.

Another point in this connection is that the amounts of the various salts added may not be an absolute indication of the actual amount of "free" salts in the different culture flasks. Undoubtedly the wood adsorbs a certain amount of the salts, but just to what extent this phenomenon takes place is undetermined. Neither is it known whether or not any one of the particular salts added is adsorbed to a greater or lesser degree than any of the others. The fact that wood is colloidal in nature may also be of some importance with reference to the removal of certain ions from the sphere of action. However, from the standpoint of practical application these effects would not be of very great importance, and it is not the purpose of this paper to go into them in detail. Attention is merely called to them in passing.

For the sake of clarity, the data presented are divided into eight series designated as follows:

1. Sodium carbonate series.
2. Sodium bicarbonate series.
3. Sodium sulphate series.
4. Sodium chlorid series.
5. Sodium carbonate-sodium sulphate series.
6. Sodium carbonate-sodium chlorid series.
7. Sodium carbonate-sodium chlorid-sodium sulphate series. Sub-series *a* and *b*.
8. Sodium sulphate-sodium chlorid series. Sub-series *a* and *b*.

## EXPERIMENTAL

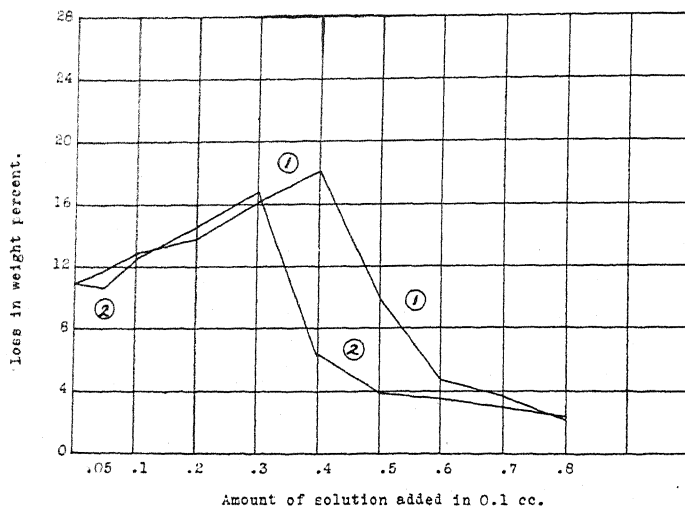
### I. Sodium Carbonate Series

In this series normal sodium carbonate solution was added to the culture flasks in increasing amounts. The usual increment of increase is 0.1 cc. except in the case of culture flasks, to which 0.05 cc. of solution was added. The results obtained from this series are recorded in table I and are plotted in curve I (text fig. 1).

TABLE I. *Effect of Sodium Carbonate on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saepiarum Fr.*

Culture No.	Salt Solution Added	Weight of Sawdust	Loss in Weight	Loss in Weight
	cc.	grams	grams	percent
1	0.05	4.70	0.55	11.7
2	0.10	4.67	0.60	12.8
3	0.20	4.62	0.64	13.8
4	0.30	4.66	0.75	16.1
5	0.40	4.63	0.84	18.1
6	0.50	4.62	0.45	9.8
7	0.60	4.64	0.22	4.7
8	0.70	4.64	0.17	3.7
9	0.80	4.66	0.10	2.1
Control. No salt solution added...	.....	.....	.....	10.9

From the data presented in table 1, it is evident that, up to a certain maximum, an increase in the amount of normal sodium carbonate solution added causes a corresponding increase in the loss in weight. After this maximum, namely, 0.40 cc. or 0.0212 g. of sodium carbonate, is reached,



TEXT FIG. 1. Curves 1 and 2. The effect of sodium carbonate (1) and sodium bicarbonate (2) on the rate of decay of Douglas fir sawdust induced by *Lenzites saeplara*.

there is a rapid decrease in the amount of decay. In other words, it would seem that the addition of small amounts of sodium carbonate, up to a maximum of 0.46 percent (based upon the dry weight of the sawdust), increases the rate of decay induced by *Lenzites saeplara*, while the addition of slightly larger amounts produces inhibitory effects. This result is a little unexpected inasmuch as *L. saeplara* is quite sensitive to the reaction of the culture medium upon which it grows.

On the other hand, it is possible that this apparent stimulation is due to the action of sodium carbonate on wood at the comparatively high temperatures existing during the process of sterilization. Small amounts of this salt may combine with the acids produced in the wood during sterilization and with the resinous substances naturally occurring in the wood. When more than 0.40 cc. of normal carbonate solution is added to the culture flasks, it is possible that more carbonate is present than will combine as above suggested, resulting in an immediate inhibition in the rate of decay. In other words the possibility is not precluded that the increased rate of decay noted in the case of those culture flasks to which small amounts of solution had been added is due to the direct action of the sodium carbonate on the wood and that inhibitory effects are produced the moment free carbonate exists. This possibility does not, however, seem to be positively supported by any of the data included in this paper.

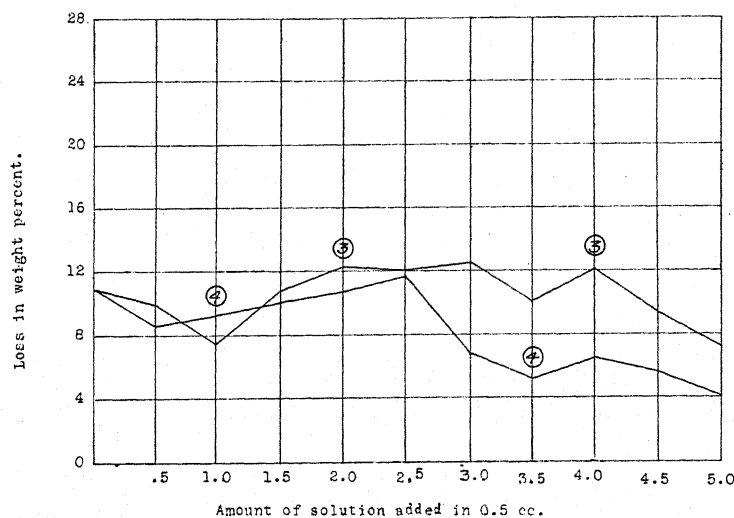
## 2. Sodium Bicarbonate Series

This series was prepared in the same manner as series 1, except that a normal solution of sodium bicarbonate was substituted for the sodium carbonate solution. The results are recorded in table 2 and plotted in curve 2 (text fig. 1).

TABLE 2. *Effect of Sodium Bicarbonate on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saeppiaria Fr.*

Culture No.	Salt Solution Added	Weight of Sawdust	Loss in Weight	Loss in Weight
	cc.	grams	grams	percent
10	0.05	4.64	0.49	10.6
11	0.10	4.62	0.58	12.5
12	0.20	4.68	0.68	14.5
13	0.30	4.66	0.78	16.7
14	0.40	4.69	0.25	5.3
15	0.50	4.63	0.18	3.9
16	0.60	4.58	0.16	3.5
17	0.70	4.65	0.14	3.0
18	0.80	4.64	0.11	2.4
Control. No salt solution added...				10.9

The similarity between curve 1 and curve 2 is immediately apparent. However, in the case of the bicarbonate an inhibitory effect is produced when more than 0.3 cc. of solution is added. In all other respects the curves are similar.



TEXT FIG. 2. Curves 3 and 4. The effect of sodium sulphate (3) and sodium chloride (4) on the rate of decay of Douglas fir sawdust induced by *Lenzites saeppiaria*.

### 3. Sodium Sulphate Series

The sodium sulphate series was prepared by adding normal sodium sulphate solution in increasing amounts to a set of culture flasks. The increment of increase in this case is 0.5 cc. and the maximum amount of solution added was 5.00 cc. The results are recorded in table 3 and are plotted in curve 3 (text fig. 2).

TABLE 3. *Effect of Sodium Sulphate on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saepiarum Fr.*

Culture No.	Salt Solution Added	Weight of Sawdust	Loss in Weight	Loss in Weight
	cc.	grams	grams	percent
19	0.50	4.65	0.46	9.9
20	1.00	4.59	0.34	7.4
21	1.50	4.68	0.50	10.7
22	2.00	4.64	0.57	12.3
23	2.50	4.66	0.56	12.0
24	3.00	4.67	0.59	12.6
25	3.50	4.64	0.47	10.1
26	4.00	4.64	0.56	12.1
27	4.50	4.64	0.43	9.3
28	5.00	4.64	0.33	7.1
Control. No salt solution added...				10.9

Although slight variations are exhibited in curve 3, it can be considered practically as a straight horizontal line curve up to a concentration of 4.0 cc., after which it shows a tendency to fall. This indicates that the presence or addition of comparatively large amounts of sodium sulphate has practically no effect on the rate of decay of Douglas fir induced by *Lenzites saepiarum*. Still larger amounts, however, exert an inhibitory effect which is no doubt osmotic in nature.

TABLE 4. *Effect of Sodium Chlorid on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saepiarum Fr.*

Culture No.	Salt Solution Added	Weight of Sawdust	Loss in Weight	Loss in Weight
	cc.	grams	grams	percent
29	0.50	4.66	0.40	8.6
30	1.00	4.65	0.43	9.3
31	1.50	4.65	0.47	10.1
32	2.00	4.65	0.50	10.7
33	2.50	4.61	0.53	11.5
34	3.00	4.67	0.32	6.9
35	3.50	4.60	0.24	5.2
36	4.00	4.67	0.31	6.6
37	4.50	4.64	0.26	5.6
38	5.00	4.67	0.19	4.1
Control. No salt solution added...				10.9

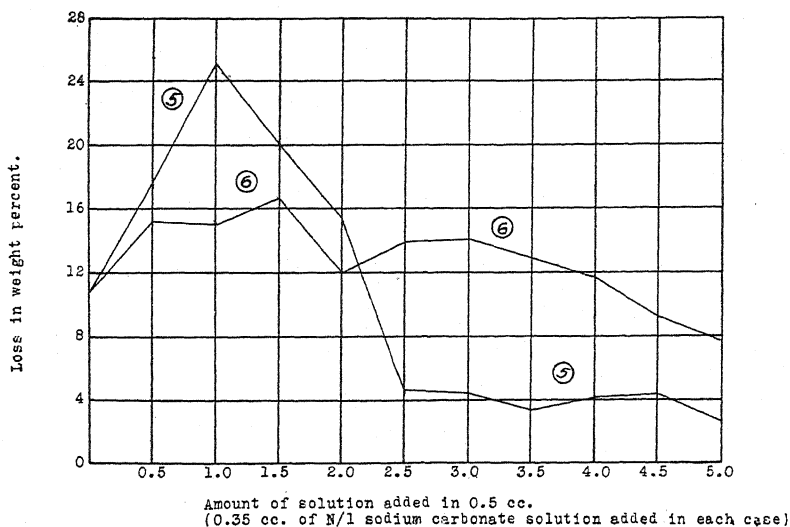
#### 4. Sodium Chlorid Series

This series was prepared in exactly the same manner as series 3, except that normal sodium chlorid solution was substituted for normal sodium sulphate solution. The results obtained from this series are recorded in table 4 and are plotted in curve 4 (text fig. 2).

Curve 4 has many points in common with the sodium sulphate curve, differing only in that it begins to fall generally after a concentration greater than 2.50 cc. is reached, while the sulphate curve fell only at a much higher concentration.

#### 5. Sodium Carbonate-Sodium Sulphate Series

Series 5 was prepared by adding to each culture flask 0.35 cc. of normal sodium carbonate solution plus varying amounts of normal sodium sulphate solution. The increment of increase in the amount of sulphate solution is 0.5 cc. as in the previous series. The results of the series are recorded in table 5 and are plotted in curve 5 (text fig. 3).



TEXT FIG. 3. Curves 5 and 6. The effect of sodium carbonate in conjunction with sodium sulphate (5) and sodium chlorid (6) on the rate of decay of Douglas fir sawdust induced by *Lenzites saepiaria*.

The data presented in table 5 are interesting particularly because the flasks to which 0.35 cc. of normal sodium carbonate solution and 1.00 cc. of normal sodium sulphate solution had been added lost more weight than any other of the culture flasks in any series. It is evident that there is a fairly rapid rise in the curve until a maximum loss in weight is reached, and then there occurs a rapid and steady decrease coincident with an increase in the amount of sodium sulphate solution added.

TABLE 5. *Effect of Sodium Carbonate and Sodium Sulphate on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saepiaria Fr.*

Culture No.	Salt Solution Added		Weight of Sawdust	Loss in Weight	Loss in Weight
	Carbon-ate	Sul-phate			
	cc.	cc.	grams	grams	percent
39	0.35	0.50	4.60	0.81	17.6
40	0.35	1.00	4.60	1.16	25.1
41	0.35	1.50	4.63	0.93	20.0
42	0.35	2.00	4.62	0.71	15.3
43	0.35	2.50	4.64	0.21	4.5
44	0.35	3.00	4.66	0.20	4.3
45	0.35	3.50	4.59	0.15	3.3
46	0.35	4.00	4.66	0.19	4.1
47	0.35	4.50	4.64	0.20	4.3
48	0.35	5.00	4.65	0.12	2.6
Control. No salt solution added. ....					10.9

#### 6. Sodium Carbonate-Sodium Chlorid Series

This series was prepared in a manner identical to that employed in the preparation of series 5, except that normal sodium chlorid solution was substituted for normal sodium sulphate solution. The data obtained from this series are recorded in table 6 and plotted in curve 6 (text fig. 3).

TABLE 6. *Effect of Sodium Carbonate and Sodium Chlorid on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saepiaria Fr.*

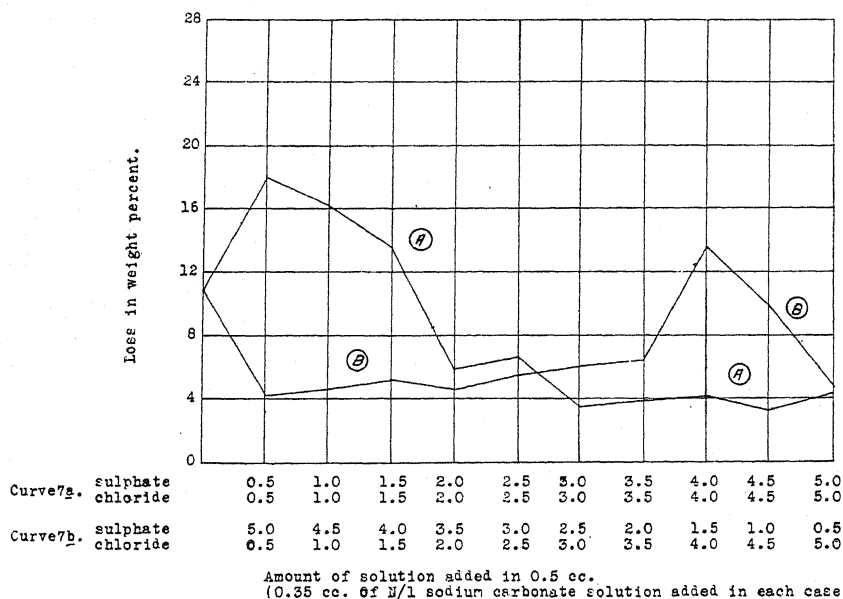
Culture No.	Salt Solution Added		Weight of Sawdust	Loss in Weight	Loss in Weight
	Carbon-ate	Chlo-rid			
	cc.	cc.	grams	grams	percent
49	0.35	0.50	4.64	0.70	15.1
50	0.35	1.00	4.60	0.69	15.0
51	0.35	1.50	4.65	0.78	16.7
52	0.35	2.00	4.56	0.55	12.0
53	0.35	2.50	4.58	0.64	13.9
54	0.35	3.00	4.60	0.65	14.1
55	0.35	3.50	4.67	0.60	12.8
56	0.35	4.00	4.48	0.52	11.6
57	0.35	4.50	4.57	0.42	9.2
58	0.35	5.00	4.56	0.35	7.7
Control. No salt solutions added. ....					10.9

The sodium carbonate-sodium chlorid curve has much in common with the sodium carbonate-sodium sulphate curve. It is evident, however, that the maximum loss in weight in the latter case is considerably less than that in the former case, and that in curve 6 the fall of the curve after the maximum had been reached is not so rapid nor is so low a general level reached as in curve 5.



### 7a. Sodium Carbonate-Sodium Sulphate-Sodium Chlorid Series

As in the two former series, the amount of sodium carbonate solution added to the individual culture flasks was the same in all cases. The amounts of sodium sulphate and sodium chlorid solutions varied as shown in table 7a. It is evident that the culture flasks to which the minimum amount of sulphate solution had been added also contained the minimum amount of chlorid solution. The data obtained from this series are recorded in table 7a and plotted in curve 7a (text fig. 4).



TEXT FIG. 4. Curves 7a and 7b. The effect of sodium carbonate, sodium sulphate, and sodium chlorid on the rate of decay of Douglas fir sawdust induced by *Lenzites saepiaria*.

TABLE 7a. Effect of Sodium Carbonate, Sodium Sulphate, and Sodium Chlorid on the Rate of Decay of Douglas Fir Sawdust Induced by *Lenzites saepiaria* Fr.

Culture No.	Salt Solutions Added			Weight of Sawdust	Loss in Weight	Loss in Weight
	Carbon-ate	Sul-phate	Chlo-rid			
	cc.	cc.	cc.	grams	grams	percent
59	0.35	0.50	0.50	4.61	0.83	18.0
60	0.35	1.00	1.00	4.70	0.76	16.2
61	0.35	1.50	1.50	4.60	0.62	13.5
62	0.35	2.00	2.00	4.76	0.28	5.9
63	0.35	2.50	2.50	4.55	0.30	6.6
64	0.35	3.00	3.00	4.62	0.16	3.5
65	0.35	3.50	3.50	4.65	0.17	3.7
66	0.35	4.00	4.00	4.64	0.19	4.1
67	0.35	4.50	4.50	4.64	0.15	3.2
68	0.35	5.00	5.00	4.60	0.20	4.3
Control. No salt solution added.						10.9

Curve 7a shows the same general relations which are shown in curves 5 and 6, namely, an upward trend where the lower concentrations are concerned followed by a fairly rapid fall as the concentration of sulphate and chlorid increases.

#### 7b. Sodium Carbonate-Sodium Sulphate-Sodium Chlorid Series

This series differs from series 7a in that the highest concentration of sodium sulphate is combined with the lowest concentration of sodium chlorid, and *vice versa*. The results obtained from this series are recorded in table 7b and are plotted in curve 7b (text fig. 4).

TABLE 7b. *Effect of Sodium Carbonate, Sodium Sulphate, and Sodium Chlorid on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saepiaria Fr.*

Culture No.	Salt Solutions Added			Weight of Sawdust	Loss in Weight	Loss in Weight
	Carbonate	Sulphate	Chlorid			
	cc.	cc.	cc.	grams	grams	percent
69	0.35	5.00	0.50	4.59	0.19	4.1
70	0.35	4.50	1.00	4.58	0.21	4.6
71	0.35	4.00	1.50	4.64	0.24	5.2
72	0.35	3.50	2.00	4.63	0.21	4.5
73	0.35	3.00	2.50	4.63	0.25	5.4
74	0.35	2.50	3.00	4.58	0.28	6.1
75	0.35	2.00	3.50	4.61	0.30	6.5
76	0.35	1.50	4.00	4.45	0.60	13.5
77	0.35	1.00	4.50	4.64	0.46	9.9
78	0.35	0.50	5.00	4.64	0.25	5.4
Control. No salt solution added.....						10.9

It is evident from the data presented in table 7b that, as concentration of sulphate decreases and that of chlorid increases, a stimulation in the rate of decay results up to a certain point. Past this point a further decrease in the amount of sulphate present with a corresponding increase in the amount of chlorid present causes a decrease in the rate of decay.

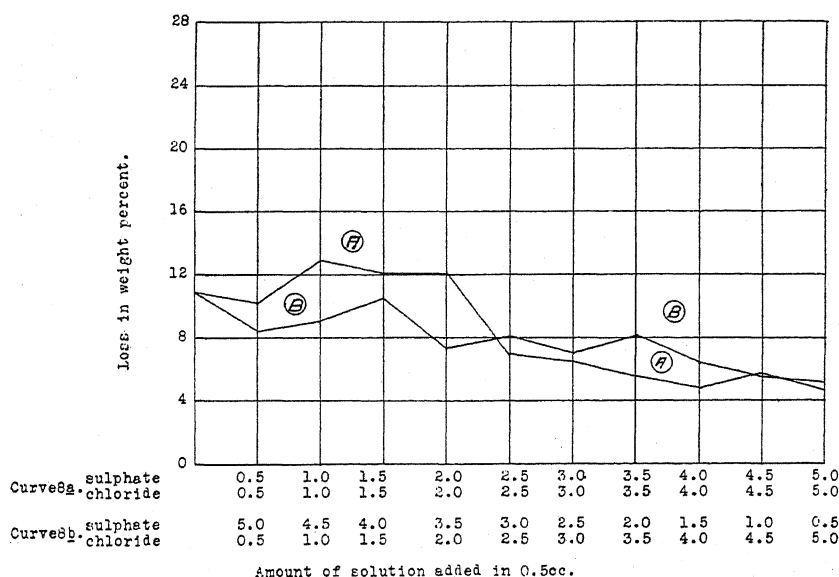
#### 8a. Sodium Sulphate-Sodium Chlorid Series

Series 8a is identical with series 7a except that sodium carbonate solution was not added to the culture flasks. The data obtained from this series are recorded in table 8a and plotted in curve 8a (text fig. 5).

It is evident that the addition in small amounts of sodium chlorid and sodium sulphate to the culture flasks has little or no effect. However, as would naturally be expected, the presence of very large amounts of these salts tends to inhibit decay on account of the osmotic conditions produced.

TABLE 8a. *Effect of Sodium Sulphate and Sodium Chlorid on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saeppiaria Fr.*

Culture No.	Salt Solutions Added		Weight of Sawdust	Loss in Weight	Loss in Weight
	Sul-phate	Chlo-rid			
	cc.	cc.	grams	grams	percent
79	0.50	0.50	4.61	0.47	10.2
80	1.00	1.00	4.66	0.60	12.9
81	1.50	1.50	4.62	0.56	12.1
82	2.00	2.00	4.61	0.56	12.1
83	2.50	2.50	4.58	0.32	7.0
84	3.00	3.00	4.62	0.30	6.5
85	3.50	3.50	4.65	0.26	5.6
86	4.00	4.00	4.66	0.23	4.9
87	4.50	4.50	4.66	0.27	5.8
88	5.00	5.00	4.64	0.22	4.7
Control. No salt solution added.....					10.9

TEXT FIG. 5. Curves 8a and 8b. The effect of sodium sulphate and sodium chlorid on the rate of decay of Douglas fir sawdust induced by *Lenzites saeppiaria*.

## 8b. Sodium Chlorid-Sodium Sulphate Series

Series 8b has the same relation to series 7b as series 8a has to series 7a. No sodium carbonate solution was added to the culture flasks of this series. The results from this series are recorded in table 8b and are plotted in curve 8b (text fig. 5).

TABLE 5b. *Effect of Sodium Sulphate and Sodium Chlorid on the Rate of Decay of Douglas Fir Sawdust Induced by Lenzites saepiarum Fr.*

Culture No.	Salt Solutions Added		Weight of Sawdust	Loss in Weight	Loss in Weight
	Sulphate	Chlorid			
	cc.	cc.	grams	grams	percent
89	5.00	0.50	4.64	0.39	8.4
90	4.50	1.00	4.61	0.42	9.1
91	4.00	1.50	4.66	0.49	10.5
92	3.50	2.00	4.61	0.34	7.4
93	3.00	2.50	4.60	0.37	8.1
94	2.50	3.00	4.66	0.33	7.1
95	2.00	3.50	4.66	0.38	8.2
96	1.50	4.00	4.61	0.30	6.5
97	1.00	4.50	4.64	0.26	5.6
98	0.50	5.00	4.60	0.24	5.2
Control. No salt solution added.....					10.9

In general, the culture flasks of this series indicate a rate of decay below the rate indicated by the control flasks.

#### DISCUSSION AND CONCLUSION

The interpretation of results obtained from decay experiments is often quite difficult. This is due principally to the fact that under apparently identical conditions the amount of decay, as indicated by the loss in weight of the flasks, often varies by wide limits. For this reason too much emphasis can not, or should not, be placed on small variations between the individual members of any series of decay experiments.

For this reason, also, it is neither the intention nor the wish of the writer to read too much into the results obtained. It may appear to many that the short discussions in connection with each series are too brief and do not cover all the points indicated by the data. This, however, is intentional.

It is felt that a conservative interpretation of the data here presented would admit of the conclusion that the presence of small amounts of sodium carbonate or sodium bicarbonate increases the rate of decay of Douglas fir sawdust induced by *Lenzites saepiarum* and that this stimulation is augmented by the presence of sodium sulphate in certain amounts. Whether or not the data indicate that the presence of sodium chlorid augments this stimulation is questionable.

This conclusion is also substantiated by the results obtained from three preliminary culture series not discussed in the present paper. In two of these series, Douglas fir sawdust was employed, and in the other, white fir sawdust. *Lenzites saepiarum* was employed as the organism causing decay in all three series. The results obtained from these preliminary series were comparable in every way to the results here recorded.

The data do not present any clear evidence that an antagonistic action between the various anions exists under the particular condition obtaining in these experiments. However, this statement should not be construed to deny the existence of such action. The experiments were not primarily designed to show antagonism between anions, and hence concentrations were not used which might have shown this phenomenon to the best advantage.

In general it also seems safe to conclude that in certain cases, at least, the average life of wood in contact with alkali soils of certain composition may be shorter than the average life of the same kind of wood in contact with ordinary soils.

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## STUDIES ON THE BIOLOGY OF *BRACHYSPORIUM TRIFOLII*

LEE BONAR

(Received for publication May 14, 1923)

In a recent paper (1920) it was shown by the writer that a hitherto undescribed fungus, *Brachysporium trifolii* Kauff., was pathogenic on white clover. During the progress of that study there appeared, in culture, what seemed to be the beginnings of perithecial bodies. This fact led to further studies in order to determine the character and further development of these bodies, and to obtain as complete data as possible regarding the physiological nature of the organism.

The genus *Brachysporium* has not hitherto been the subject of very much laboratory study. On the genus *Helminthosporium*, however, to which *Brachysporium* is very closely allied, a considerable number of papers have appeared, especially along pathological lines, as well as on life-history studies for determining the ascus stage of a number of forms (Hecke, 1898; Ravn, 1901; Diedecke, 1902-1903; Noack, 1905).

The present paper is a record of the studies which were made in an attempt to learn the complete life history of the fungus, and in this way to interpret the significance of the above-mentioned bodies formed in some of the cultures.

This work has been carried on in the laboratories of the Department of Botany at the University of Michigan, under the direction of Prof. C. H. Kauffman to whom are due my whole-hearted thanks for aid and encouragement at all times.

### HISTORICAL REVIEW

The intensive study of fungi in culture has long been a subject of interest to mycologists. The method of approach of different workers has varied greatly, both as to point of view and as to methods of study employed.

The first comprehensive discussion of this subject is given by Brefeld (1881) in his "Culturmethoden zur Untersuchung der Pilze." In this discussion he points out many of the problems with which one meets in the growing of fungi. He gives a summary of the methods employed in his

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own studies, and to some extent of those used by de Bary. These men used, for the most part, either sterilized natural products or extracts or decoctions of these, as substrata for their fungi. They have a very large following among present-day pathological and mycological workers. In many cases satisfactory growth and reproduction have been secured on substrata containing such natural products; but as to the nature and composition of these we can make only rough estimates. Likewise, the growing of fungi in cultures where the food content is definitely known has been practised to a considerable degree. The use of definitely synthesized solutions has been the means of affording us much valuable and definite information.

Pasteur (1858), in his studies on the organisms that cause fermentation, was able to demonstrate the definite rôle played by microorganisms in the chemistry of fermentation. Raulin (1869) published records of the cultivation of fungi on solutions of organic and inorganic salts. Nägeli (1880) contributed a large amount of data on the growth of yeasts and bacteria in synthetic solutions.

The use of some natural products in combination with pure chemical substances of some sort has been very widely employed by a large number of workers during recent years.

The idea of the possibility of finding a universally favorable medium for growth and reproduction in fungi, although often not expressed in a definite statement, seems to have permeated a great deal of the work of recent years. A careful comparison of the physiological reactions of any small number of different organisms will argue very strongly against any such possibility.

During the last decade of the past century there seems to have come a great wave of activity in the study of filamentous fungi in culture. Probably the greatest activity was in the working out of life histories of different forms, especially in the connecting of the ascus and the imperfect stages. Continual use was made of various natural products as food, of synthetic media, and of combinations of these. There came to be a great deal of attention given to the physical factors affecting the development of the organism, such as light, temperature, oxygenation, etc. The chief early exponent and leader in this line of work was Klebs who, in 1896, published his work on algae and fungi, calling attention to the very important rôle played by the external controllable factors in the morphogenesis of the plant and of its organs. The general application of these principles has been frequently demonstrated by workers who have applied them to many different forms and groups of fungi. Beijerinck (1901) showed that the use of food materials of definite composition might be employed as the basis for selective separation of bacteria in mixed cultures. This principle has been very generally used in recent years in bacteriological work. Bachmann (1895) showed the effect of external conditions on spore-formation in *Thamnidium elegans* Link. Klebs (1899, 1913) published comprehensive

summaries of the facts and principles in this field of work, adding new facts and giving an interpretation of the facts that had accumulated. Leininger (1911), working with *Pestalozzia palmarum* Cke., showed that, by the manipulation of the conditions in the culture, one and the same type of spore might be produced either in a pycnidium, in a pseudopycnidium, or on free-growing hyphomycetous conidiophores.

The Saprolegniaceae have been carefully studied by a number of workers; by Klebs (1899), with a single strain of *Saprolegnia mixta*, and by Kauffman (1908, 1921) with different strains of *Saprolegnia mixta* as well as with other species of the family. Kauffman was able to show that the growth and reproduction of these different forms may be controlled by very definite external conditions, and that the potentialities for the development of each form are specific under a given set of conditions. Pieters (1915) brought out the fact that there is no necessary relation between growth and reproduction in the Saprolegnias, and that each process is dependent on a definite set of conditions which are not constant for different forms.

Stevens and Hall (1909) demonstrated that there is a wide range of variation in characters ordinarily used in taxonomic work, when the fungi are subjected to varying conditions of growth in culture. They used a goodly number of forms representing widely divergent groups of plants. They considered the density of the colonies in the culture, the density of the mycelium in relation to zonation on agar media, the chemical composition of the food, and the action of light affecting the cultures.

Coons (1916), by a very intensive study of *Plenodomus fuscomaculans*, showed that in the Sphaeropsidales the physiological reactions of the plants are just as definite and exact as in the groups earlier studied. He gave an especially interesting demonstration of the effect of light on reproduction, and of the nature of this light effect, by showing it to be an oxidation process. Harter, Weimer, and others (1918, 1921) made an application of these principles concerning the effect of varying conditions on the growth of fungi, in their study of the storage rots of the sweet potato. They considered a large number of different sets of conditions to be the optima for the development of different fungi. Their recommendations for preventive measures are based very largely on the idea of an avoidance of these conditions. Leonian (1921), in working out the life history of *Valsa leucostoma*, was able to show the determinate relation of the food material to the production of the ascus-bearing stage of the fungus.

#### THE FUNGUS, ITS MORPHOLOGY AND CYTOLOGY

A detailed description of *Brachysporium trifolii* Kauff. was given in an earlier paper (Bonar, 1920), but a brief résumé may be given here. This fungus attacks the petioles and leaves of the clover plant, appearing as erect brown conidiophores arising from the mycelium within the tissue of the host. The conidiophores bear from one to several spores at their tips.

The spores are usually 3-septate, with the antepenultimate cell, in most cases, enlarged on one side, thus giving the spore a more or less triangular shape. The spores are dark fuscous brown, and measure  $21-31 \times 9-11$  microns.

A correction in the former description of the fungus should be made at this time. In that publication, a second spore form belonging to the genus *Blennoria* was described as a part of the life history of the *Brachysporium*. It was impossible to isolate this form at that time, and hence it had not been reinoculated into the host to check the evidence for connection. In December, 1921, this *Blennoria* was again found in the greenhouse, this time on plants of the white clover which had not been inoculated with *Brachysporium*. Isolation cultures were made, and the fungus was found to be distinct from *Brachysporium*. Inoculation of clover plants, by spraying with a spore suspension from a pure culture of the fungus, resulted in an infection on about ten percent of the leaves of the plants so treated. Those leaves which were attacked wilted completely, and the typical acervuli appeared on these dying parts which became brown in color. In this manner the fungus, which belongs to the genus *Blennoria* of the *Melanconiaceae*, was shown to be a weak parasite on white clover under greenhouse conditions. This fungus, therefore, must receive an autonomous name, and the combination *Blennoria trifolii* is hereby proposed. For description see the paper referred to (Bonar, 1920, p. 441 and fig. 2, p. 438).

*Brachysporium trifolii*, when grown on favorable nutrient substrata such as cornmeal agar or on various synthetic agars containing sugars and chemical salts, grows rapidly, and after a few days produces an abundant growth of grayish mycelium. This, as a rule, produces a floccose aerial growth. The typical conidiophores and conidia form in great abundance; frequently, however, the conidiophores are abortive. Conidia are formed in about 6-8 days on the more favorable media. As the conidiophores and spores begin to form, the cultures become darker, in consequence of the darker color of the conidiophores, the spores, and the older massed mycelium.

In cultures on oatmeal agar (formula of Pethybridge and Murphy, 1913) between two and three weeks old, small black bodies appear on the surface of the medium. These grow upward through the mass of aerial mycelium in the form of black columnar stalks. They are a dense black, except the rounded growing tip which is often almost hyaline. The stalks often fork one, two, or three times into an indefinite number of branches. The branching sometimes seems to be dichotomous, while in other cases it is irregular. The bodies may arise singly or in groups, and may be branched or unbranched. They vary in height up to 10 mm., averaging about 6 mm. when developed on oatmeal agar in petri dishes or test tubes. The bodies are at first smooth and are composed of a compact, stroma-like tissue free from any hyphal outgrowths. The older bodies, however, sometimes

become tufted near the apex with brown hyphae. These hyphae, in turn, may bear conidia, or may form simple, hairlike outgrowths (Pl. II, figs. 1a, 1b).

These bodies grow steadily after their appearance in a petri-dish culture, until, after 30-40 days, they present an appearance not unlike a miniature forest set thickly over the surface of the medium. The appearance of these bodies in such abundance on oatmeal agar alone, out of a variety of different media, is a very significant fact. It shows a very marked reaction to the special quality of the food in this substratum. A more careful examination of these bodies shows that they arise from the upper portion of the agar layer, where there has developed an interwoven black mass of hyphae which, however, is not a definite stroma. In section, these younger bodies are seen to consist of pseudoparenchymatous tissue. The outer two or three layers of cells, more especially those of the outer layer, have thick, blackened walls, while the interior tissue is hyaline, with cell walls very like those of the vegetative mycelium in thickness.

The very early stages, when examined in free-hand sections, fail to show any differentiation of tissue in the interior except the differentiation of the black outer wall from the inner hyaline portion. For the further work, paraffin sections were therefore made. A number of different killing fluids and staining methods have been used. The use of Flemming's weak solution and of the triple stain was settled upon as the best treatment for the material in hand. A study of these stained preparations made from the young bodies, 30-35 days old, shows them to be made up of homogeneous tissue. This tissue is composed of very closely interwoven hyphae; in a thin section the cells appear very irregular in shape (figs. 2-4). These cells are also very irregular in size, and those of the first-formed, basal portion are often much larger than those farther up in the body. This tissue arises from a closely interwoven mat of more or less horizontal mycelium in the upper layer of the agar medium, as is shown in figure 2. As emphasized before, this mat is not a true stroma, and, in sections 10 microns in thickness, individual hyphae can be traced for quite a distance through the agar. In other words, they are merely stromatic strands. Preparations made from cultures 45 days old, and from others 60 days old, show, in their interior and a short distance below the tip, a very distinctly differentiated area (fig. 6). The cells within this area are much larger, more loosely connected, and thinner-walled than those of the rest of the body (compare figs. 7 and 8). Furthermore, this area is bounded by a distinct layer of elongated, narrow, very closely packed cells giving the impression of a definite wall around this inner area (Pl. III, fig. 12). In most cases the wall extends completely around this egg-shaped or ellipsoidal area, but in some instances it extends only a part of the way around. In such cases it is dome-shaped above, while at its lower end there is a gradual transition from the enclosed area of the larger differentiated cells to that of the homogeneous

cells which are characteristic of the tissue of the body as a whole. No sharp line or delimiting wall is found, as one moves his point of observation from the inner area downward into the homogeneous body tissue.

If we examine now the cells of the developing columnar bodies in detail, some unusual characteristics appear. The cells of the very young bodies are filled with protoplasm. When this is stained each cell is found to contain from 1 to 5 nuclei, with strands of cytoplasm radiating out from them (Pl. II, figs. 4a, 4b). These nuclei are very small and little can be learned as to their structure. In very clear preparations there is seen within the nucleus a small globular body which is considered a nucleolus. This small particle takes a very brilliant safranin stain, while the remainder of the nucleus is less deeply stained and slightly granular in appearance. As the material becomes older, there is a disappearance of this cell protoplasm until in some cultures 45 days old, and regularly so in 60-day cultures, the compartments stand out as mere walls, void of all cell contents (fig. 7). This disintegration and disappearance of the protoplasm is complete in a great many of the bodies. In some, however, there are found, at this stage, single cells or small clumps of cells which take a very deep stain and hold this stain until all the surrounding tissue is completely destained. These deeply staining cells are found to be considerably larger on the average than those of the surrounding homogeneous tissue. These special cells or clumps of cells may be found singly or scattered through the upper portion of the stalked body. An extensive examination fails to show any connection between the different points of differentiation in a single body where there are such. A great many of these special cells have been examined in an effort to understand the significance of their differentiation. Cases as represented in figures 9a-9c, Plate III, have been found. These show the connection and relation of these larger deeply staining cells with the cells of the homogeneous surrounding tissue. From these figures it is evident that the special cells arise by a differentiation from the ordinary body cells. As before stated, the cells of the body as a whole all contain an abundance of protoplasm when the body is young. Then comes a time in its history when a disintegration of this cellular protoplasm sets in. At special loci, however, the determinant of which we do not know, an exception to this general process occurs. Certain cells are found in or on these intertwined hyphal strands which continue to show an abundance of protoplasm which has a very marked staining reaction. These cells stand out, in thin preparations, in sharp contrast to the surrounding tissue. These differentiated cells by growth and multiplication form clumps of similar cells, as is shown in figures 9a, 9b, and 9c. By continued growth one of these clumps enlarges until we have a stage such as is represented in figure 10. Here can be seen a large number of these cells which are sharply differentiated from the surrounding tissue. Around this specialized area, in section, can be seen a band of narrow, irregularly elongated cells which tend to set this specialized area off from the outer tissue.

These apparently are ordinary body cells which have become flattened and more or less collapsed from the pressure of the growing, expanding group of cells within. The result of this is that a zone of these elongated cells is formed around the inner growing area, and this zone forms the delimiting wall such as is shown in figure 12 in an older case. The growth and expansion of this inner area continues until a certain degree of development is reached or a certain set of conditions prevail, beyond, or on account of, which, further differentiation does not occur. Instead of further growth, a process of disintegration then sets in within the protoplasm of the cells in this area. In figure 11 are seen some cells which contain their normal nuclei and cytoplasm, while in others there has been a disintegration so that the cell is empty, in part. In still other cells it will be seen that there has been a complete disappearance of the protoplasm, and the cell wall alone remains. In older material large numbers of the differentiated areas are found, within which the cells are completely lacking in any protoplasmic content (fig. 12). These empty cells of the differentiated area are very different, however, from those which make up the surrounding general tissue of the stalked body as a whole. This is best brought out by a comparison of figure 7 with figure 8 (Pl. II). These figures are both taken from the section shown as a whole in figure 12. Figure 7 is an enlarged view of a portion of the tissue outside the central differentiated area. Figure 8 is an equal magnification of a portion of the inner area and is separated from that of figure 7 by the wall, only a few cells in thickness.

The time, in the life history of a culture, of the beginning of these differentiated areas in the stalked bodies is rather uncertain. Material from 30-day cultures does not show any differentiation, and most of the cells of the stalked body show stainable protoplasmic contents. Material from 45-day cultures shows specialized deeply staining cells or small clumps of these cells. Figure 10 was made from material from a 4-months-old culture, but other preparations from 4-months-old cultures have been studied which show the degree of development represented in figure 12. This process is apparently very much affected by the conditions under which the cultures are developed, as are the other life processes of this organism.

#### CULTURE METHODS AND MEDIA

*Brachysporium trifolii* has been grown on a large number of different kinds of media. Methods for their preparation and the formulae are given below. What are spoken of as agars were used in test tubes and petri dishes. For solution cultures, 25-cc. loose-cover glass capsules each containing a cone of filter paper, such as were described by Coons (1916), were used at first. The most favorable cultures were secured, however, by the use of filter-paper cups in capsules, as described by Leonian (1921). Into these filter-paper cups—made by pushing in the moistened tip of a filter-paper cone with the finger—any kind of food materials could easily be placed; as,



for example, agars, corn meal, wheat flour, gluten, rice, or rice paste, which were used in this way. These cultures were prepared and then sterilized in the autoclave. The water supply was kept constant by keeping sterile water in the bottom of the capsule. The filter paper served as a wick to supply a constant moisture to the material in the cups. Genuine Whatman no. 1 filter paper, made by W. and R. Ralston, was used for all these cultures. Distilled water was used for all preparations.

The media were prepared as follows:

Melilotus stems: green stems of *Melilotus* cut in sections 3 inches long. Put in test tubes with a little water, and autoclaved at 15 lbs. for 20 min.

Green beans: green string-bean pod put in test tube with a little water, autoclaved at 15 lbs. for 20 min.

Potato plugs: cylinders of fresh potato in test tube with a little water, autoclaved at 15 lbs., 20 min.

Carrot plugs: same as potato plugs.

Rice grains, rice paste, wheat flour, gluten, pure wheat starch, and corn meal: put material in filter-paper cups in capsules with a little water in bottom of capsule, autoclaved at 15 lbs., 20 min.

Cornmeal agar: 4 teaspoonfuls cornmeal, 1000 cc. water, cook in double boiler at 60° C. for 1 hour. Strain through gauze and add to filtrate 15 g. agar: heat until agar is dissolved. Filter through filter paper, autoclave at 15 lbs., 20 min.

Prune-juice agar: cook 200 g. prunes in 500 cc. water slowly for 1 hr. Restore to original volume, add 500 cc. water in which is dissolved 15 g. agar. Clear with white of egg, filter, autoclave at 15 lbs., 30 min.

Duggar's agar (Duggar, "Fungous Diseases of Plants," p. 26):  $\text{NH}_4\text{NO}_3$  1.0 g.,  $\text{MgSO}_4$  0.25 g.,  $\text{KH}_2\text{PO}_4$  0.5 g.,  $\text{FeCl}_3$  trace, cane sugar 5.0 g., water 100 cc., agar 1.5%. Dissolve, clear, and filter through filter paper. Autoclave at 15 lbs., 15 min.

Duggar's agar and peptone: same as above, plus 0.2% peptone.

Oatmeal agar or "oat agar": grind 60 g. Quaker oats to fine powder. Stir into 1000 cc. cold water. Cook in double boiler with frequent stirrings 10 or 15 minutes. Add 10 g. agar. Continue heating until agar is dissolved. Stir constantly. Pour into large test tubes and autoclave at 15 lbs., 45 min.

Light oat agar: same as above except that 30 g. oatmeal is used instead of 60 g.

Water extract of oatmeal: 60 g. oatmeal in 1000 cc. water. Let stand in cool place for 24 hrs. with occasional stirring. Strain through cloth, filter through paper, autoclave at 15 lbs., 20 min.

Leonian's agar: water 1000 cc.,  $\text{KH}_2\text{PO}_4$  1.25 g.,  $\text{MgSO}_4$  0.625 g., maltose 6.25 g., malt extract 6.25 g., agar 15 g. Dissolve salts in 500 cc. of water. Dissolve agar in other 500 cc., mix the two, clear, filter, autoclave at 10 lbs. for 15 min.

Leonian's solution: use solution as above without agar.

Coons' solution: use as materials M/5 solutions. For 100 cc. nutrient solution, take  $\text{MgSO}_4$  1 cc.,  $\text{KH}_2\text{PO}_4$  5 cc., asparagin 5 cc., maltose 5 cc., water 84 cc., autoclave at 10 lbs., 15 min.

Modification of Coons' solution: same as above, except that M/5 levulose is substituted for M/5 maltose.

Agars plus carbohydrates: to the prepared and melted agar is added, and intimately mixed, before sterilization, the desired percentage of the carbohydrate.

Solution e:

Dihydrogen potassium phosphate	M/20 sol., 50 cc.
Calcium nitrate	M/20 sol., 50 cc.
Levulose	4 percent, 50 cc.
Magnesium sulphate	M/20, 2 cc.



Solution *f*: Solution *e*, using 10 percent levulose instead of 4 percent.

Solution *g*: Solution *e*, plus 50 cc. of 4 percent malt extract.

Solution *h*: Solution *g*, plus 50 cc. 0.2 percent peptone.

Solutions, *e*, *f*, *g*, and *h*, as well as various sugar solutions, were autoclaved at 10 lbs. for 15 min.

In the sterilization of media I followed the idea put forward by Mudge (1917), that a high temperature for a short time has less effect in hydrolyzing proteins and sugars than does a lower temperature for a longer time.

### EXPERIMENTAL STUDIES

The bodies which have been previously described under "Morphology and cytology" appeared early in the study of the fungus. The first cases of differentiation observed were of a stage such as represented in figure 12, Plate III. The question of their significance in the life history of the organism was not easily answered. The most logical conclusion to be drawn at that time was that these differentiated areas represented an incomplete development of some reproductive body, possibly a perithecium. As a starting-point, a number of experiments were set up to analyze the following factors: first, conditions which might promote further development in these differentiated areas; second, the presence of possible inhibitory influences, which might arrest a further differentiation in these areas; and third, specific physiological reactions of the plant toward controlled environmental conditions. Data obtained from these experiments have been arranged according to: (1) vegetative or mycelial growth; (2) conidium-production; (3) the production of the stalked bodies. These data are given where possible in tabular form by the use of symbols to represent degrees of development. The Roman numerals, O, I, II, III, IV, and V are employed to represent varying degrees of development. O, for example, means no development, and V the maximum development of the process under consideration.

Tables 1, 2, and 3 classify these substrata with regard to the degree of development which they induce in each of the above-named processes of the plant. The factors influencing these different processes are discussed separately following each of the three tables.

### FACTORS INFLUENCING MYCELIAL GROWTH

#### Physical Factors

##### *Temperature*

One of the most important factors in the growth of any plant is temperature. Mention is made of the effect of temperature on the growth of this fungus in the earlier publication (Bonar, 1920). Later checking up of these results and records of further study are given here. The lower limit for growth is between 6° and 8° C. Growth is slow and long-continued between

8 and 16 degrees. Growth is much more extensive and rapid at a higher temperature up to its optimum, which is between 20° and 24° C. As the temperature is raised above 24° C. (providing humidity is favorable), the growth gradually becomes less extensive, until at 30° C. 1-month-old cultures show a small growth colony 1 cm. in diameter on the more favorable media.

TABLE I. Degree of Vegetative Growth in Different Substrata

O	I	II	III	IV	V
	Sol. <i>e</i> and M/1 NaCl	Gluten	Pot. plugs	Melilotus stems	Pure wheat starch
	Sol. <i>e</i> and 2M NaCl	Beef-infusion agar	Green beans	Whole rice	Cornmeal
	Oat-agar cup and 2.5M NaCl	Duggar's agar and 0.2% peptone	Carrot plugs	Wheat flour	Cornmeal agar
	Oat-agar cup and 3M NaCl	Water extract of oatmeal	Rice-flour paste	Pot. agar	Prune-juice agar
		Maltose 1%	Duggar's agar	Light oat agar	Leonian's agar
		Glucose 1%	Oat agar, 10% agar	Pot. agar and 1% levulose	Oat agar
		Oat agar and 1.5 M NaCl and 1% lev.	Glucose 5%	Pot. agar and 1% sucrose	Oat agar and 1% sucrose
		Oat agar and 2M NaCl and 1% lev.	Levulose 1%	Pot. agar and 1% glycerin	Oat agar and 1% levulose
	Sol. <i>e</i> and M/2 NaCl		Sol. <i>f</i>	Oat agar and 1% glycerin	Oat agar and 1% glucose
			Sol. <i>h</i>	Oat agar and 1% lev. and 1% glycerin	Oat agar and 1% maltose
			Coon's sol.	Sol. <i>e</i>	Cornmeal agar and 1% levulose
			Plain agar cups and Coons' sol.	Sol. <i>g</i>	Light oat agar and 1% lev.
			Oat agar and M/1 NaCl and 1% lev.	Mod. of Coons' sol.	Leonian's sol.
			Sol. <i>e</i> and M/5 NaCl	Oat agar and $\text{KH}_2\text{PO}_4$ and 1% maltose	Oat agar and $\text{KH}_2\text{PO}_4$ and 1% lev.
				Oat agar and Coons' sol.	Oat agar and sol. <i>e</i>
				Oat agar and mod. Coons' sol.	Oat agar and $\text{KH}_2\text{PO}_4$
				Plain agar cups and sol. <i>e</i>	Oat agar and $\text{NaH}_2\text{PO}_4$
				Plain agar cups and mod. of Coons' sol.	Oat agar and sol. <i>e</i> — $\text{MgSO}_4$
				Oat agar and M/5 NaCl and 1% lev.	Oat agar and M/2 NaCl and 1% lev.
				Oat agar and M/2 NaCl and 1% lev.	Oat agar and M/10 NaCl and 1% lev.
				Sol. <i>e</i> and M/10 NaCl	

The effect of temperature on the time of spore germination is very marked, as is shown in the following experiment. Spores were placed in hanging-drop cultures in water, in Van Tieghem cells, and these were subjected to different temperatures. The length of time necessary for the formation of a well differentiated germ tube was noted.

Spores at 0° C.	No germination after 8 days.
" " 10° C.	Germinated in 18-36 hours
" " 22° C.	" " 4-9 "
" " 25° C.	" " 3-5 "
" " 30° C.	" " 2-3 "

The time given represents the range from the time when any single spore was seen to be germinating until more than 50 percent of the spores in the suspension had been germinated. Spores kept 8 days at 0° C. germinated in 1 to 2 hours when transferred from 0° C. to 30° C.

### *Light*

In the study of this point, different types of cultures were grown in diffuse light and in the dark for varying lengths of time, but no marked difference could ever be detected between them. Cultures grown by a window, where they were subjected to the direct rays of the sun for a few hours each day, produced less extensive growth of mycelium than those in diffuse light or in the dark.

### *Oxygen Requirements*

The mycelium of this fungus requires an abundant supply of oxygen for growth. This is shown by the following experiment. Test-tube cultures when three days old, showing a tuft of vigorously growing mycelium, were sealed by dipping the plugs into melted paraffin. Cultures of the same series were kept unsealed as checks. The growth continued in the sealed tubes for 1 to 2 weeks, and in that time did not cover the agar slant in the tube. After two weeks no perceptible growth occurred in the sealed tubes, while the cultures in the unsealed tubes continued to grow and covered the whole of the surface of the slant with a heavy mat of mycelium.

### *Humidity*

The degree of humidity in the culture chamber has a very evident effect on growth. In the preliminary report it was stated that no growth was secured at 26 degrees C. The medium then used was oatmeal agar in petri dishes, placed in an incubator. Further studies on this point reveal the fact that in a dry atmosphere growth ceases at from 26 to 28 degrees C., while in an atmosphere approaching saturation, and on favorable substrata, growth may occur at higher temperatures. Cultures on filter-paper cones, set with their bases in a nutrient solution in loose-cover capsules, show a slight growth at 30 degrees C. These colonies are from 1 to 2 cm. in diameter at the end of one month, and the mycelium is composed, in large part, of irregular clumps of heavy-walled, dark, rounded cells.

### Physico-chemical Factors

There are certain classes of chemicals, the presence or the absence of which, although they do not act as foods, may affect the growth of the fungus in culture in a very decided manner. Klebs (1899) makes the generalization that fungi as a rule grow best on slightly acid media. Coons (1916) says: "It is commonly said that fungi grow best under slightly alkaline conditions." It matters little whether we try to make generalizations or not, we must always consider the specific requirements of the organism under consideration, and these will frequently be found to be different from the requirements of many other organisms. The plant here studied shows a rather wide tolerance to either acidity or alkalinity in relation to its vegetative growth. A number of titrations made on oatmeal agar, which is the optimum substratum for this fungus, show an average reaction<sup>1</sup> of + 1. Titrations on a number of the other media, as they are made up, show them as a rule to be slightly acid in their reaction.

To test the reaction of the plant under study, five sets of cultures on oatmeal agar were made up, in which the reaction was adjusted as follows: - 1, neutral, + 1, + 2, and + 3. (In the last-cited the agar failed to jell because of the excess of acid.) Growth in -1 was in the form of a thin, closely packed felt over the surface, and was of slight amount; in neutral there was very much more growth with a fluffy aërial mycelium; in + 1, which proved to be the optimum, a very vigorous and extensive growth occurred; in + 2 the growth was very like that in neutral, while in + 3 it was very much reduced but slightly more extensive than in -1. Thus it is shown that this fungus, while not very sensitive to slight changes in acidity, grows best on a slightly acid substratum.

### Quality of Food

This fungus grows, as is shown in table 1, on a very large number of different kinds of substrata. It is seen from this table also that a favorable degree of vegetative growth occurs on a large number of these. Most important for vegetative growth, it seems, is the presence of a plentiful supply of carbohydrate material, as will be seen from a study of column V of the table. There are, however, specific relations between the extent of growth and the nature of the different carbohydrates used as food. Pure starch from wheat gives a very abundant growth. 1 percent solutions of glucose or maltose do not produce so extensive a growth as does a solution of levulose of like concentration. Those natural products like cornmeal, oatmeal, and rice, or products of these, contain, besides an abundance of carbohydrates, a supply of proteins and certain salts, and all produce a vigorous

<sup>1</sup> The acidity or the alkalinity of the medium is expressed in terms of that quantity, in cc., of a normal (N/1) volumetric solution which would bring 100 cc. of it to the neutral point, using phenolphthalein as an indicator. If acid, the plus sign (+) is placed before the number expressing this amount; if alkaline, the minus sign (-) is used instead.

growth when used as media. Those substances containing a high percentage of nitrogen with a low carbohydrate content are unfavorable for vegetative growth. Examples of these are beef-infusion agar, which contains an abundance of animal protein with very little carbohydrate material, and gluten, which contains only the proteins from wheat flour. The specific nature of the nitrogenous matter is also effective in influencing growth. Leonian's solution, having as its nitrogen supply malt extract, is much more favorable for growth than Coons' solution, in which asparagin is used. An appreciable reduction in growth occurs when to Duggar's agar is added 0.2 percent peptone. These different media all contain the salts which are necessary for growth, but the chief factors influencing the amount of growth seem to be combinations of certain carbohydrates and proteins.

### Quantity of Food

The quantity of food is a less important factor than is the quality of food, in the production of mycelial growth in this fungus. All the media used are comparatively rich and far above the concentration minimum for growth. In a study of table I, however, there appear some instances in which the quantity of the food shows an appreciable effect. A 1 percent solution of glucose gives a growth of II, while a 5 percent solution gives a growth of III. Water extract of oatmeal gives a growth of II, while oatmeal gives a growth of V. The osmotic concentration of the nutrient solution is also an important factor. Solution *f* gives a growth of III as compared to a growth of IV for solution *e*. The two solutions are identical except in the percentage of levulose present. In solution *e*, 4 percent levulose is used. In solution *f*, 10 percent levulose is used. Other examples of the effect of concentration are seen in those cultures to which varying amounts of NaCl have been added. Oatmeal-agar cups to which had been added 1 percent levulose solution were used as checks in these experiments. Varying amounts of NaCl were added to this levulose solution. When NaCl is added to the levulose solution in quantities to make an M/20 or M/10 solution of NaCl, the amount of growth is not affected. When more than these amounts are added there is effected a reduction in growth until in 2 M or 3 M solution the growth is represented by I. In such extreme cases the growth of the fungus is almost entirely composed of heavy-walled, rounded chlamydospores in irregular clumps or masses. Growth in nutrient solution *e* is more seriously affected by the addition of NaCl than are those cultures on agar. Cultures on solution *e* show a reduction of growth in concentrations of M/1 to 2 M NaCl equal in degree to that shown in 2 M to 3 M solutions which have been added to oatmeal agar in cups. The nature of the growth in these solutions varies from a much-branched clear mycelium in solution *e*, or solution *e* plus NaCl to make an M/10 solution, to irregular clumps of thick-walled, black chlamydospores in M/1 or 2 M solutions of NaCl in solution *e*.

TABLE 2. *Degree of Conidium-production on Different Substrata*

O	I	II	III	IV	V
Potato plugs	Pot. agar and 1% glycerin	Green beans	Carrot plugs	Wheat flour	Melilotus stems
1% maltose	Oat agar and 1% lev. and 2 M NaCl	Gluten	Whole rice	Pure wheat starch	Cornmeal
1% glucose	Oat agar and 1% lev. and 2 M NaCl	Sol. f	Rice-flour paste	Sol. e	Cornmeal agar
1% levulose	Sol. e and M/2 NaCl	Leonian's agar	Sol. g	Prune-juice agar	Oatmeal agar
5% glucose	.	Duggar's agar and 0.2% peptone	Sol. h	Light oat agar	Oat agar and 1% lev.
Beef-infusion agar		Water extract of oatmeal	Coons' sol.	Oat agar and 1% glucose	Oat agar and 1% sucrose
Oat agar and 1% lev. and 2.5 M NaCl			Mod. of Coons' sol.	Light oat agar and 1% lev.	Cornmeal agar and 1% lev.
Oat agar and 1% lev. and 3M NaCl		Pot. agar and 1% lev.	Pot. agar	Oat agar, 1% maltose, and $\text{KH}_2\text{PO}_4$	Oat agar and 1% lev. and M/20 $\text{KH}_2\text{PO}_4$
Sol. e and 1.5 M NaCl		Pot. agar and 1% sucrose	Duggar's agar	Oat agar, Coons' sol.	Oat agar and mod. of Coons' sol.
Sol. e and 2 M NaCl		Oat agar and 1% lev. and M/1 NaCl	10% oatmeal agar	Oat agar and sol. e — $\text{MgSO}_4$	Oat agar and sol. e
		Sol. e and NaCl	Oat agar and 1% maltose	Plain agar cups and Coons' sol.	Oat agar and M/20 $\text{KH}_2\text{PO}_4$
			Oat agar and 1% glycerin	Plain agar cups and mod of Coons' sol.	Plain agar cups and sol. e
			Oat agar and 1% lev. and 1% glycerin	Oat agar and 1% lev. and M/5 NaCl	Oat agar and M/20 $\text{NaH}_2\text{PO}_4$
			Oat agar and 1% lev. and M/2 NaCl	Sol. e and M/10 NaCl	Oat agar and 1% lev. and M/20 NaCl
					Oat agar and 1% lev. and M/10 NaCl

## FACTORS INFLUENCING CONIDIUM-PRODUCTION

Table 2 classifies the various substrata with reference to the degree of conidium-formation they induce. The estimates of the degree of conidium-formation are based on the average number of spores found in a number of fields under the microscope, using as nearly as possible uniform bulk of material and uniform dilution in preparing the amount.

### Physical Factors

#### *Temperature*

The optimum temperature for conidium-production is the same as that for growth, 20°–24° C. The minimum temperature for conidium-production is 15°–16° C. The maximum is, under other optimum culture conditions, 27° C. The cultures used for the compilation of the tables were grown at the optimum temperature of 22°–25° C.

#### *Light*

No perceptible effect on the production of conidia is produced by keeping in either the light or the dark.

#### *Oxygen Requirements*

Conidium-production is reduced, by the exclusion of oxygen, to even a greater degree than is mycelial growth.

#### *Humidity*

The humidity of the culture chamber influences the amount of conidium-production to a fairly marked degree. In cultures where the atmosphere is very dry, production is inhibited. In cultures which afford a moist substratum, such as Melilotus stems, there is a very abundant conidium-production with a comparatively low degree of moisture in the culture chamber. In culture chambers which approach saturation, fewer conidia are produced; here the conidiophores are not formed in a regular manner, but a fluffy aerial mycelium develops with abortive conidiophores.

### Physico-chemical Factors

Acidity or alkalinity of the culture medium is also a factor in the production of conidia.

Cultures with reaction of  $-1$  are marked O for conidium-production.  
Cultures with reaction of 0 are marked III for conidium-production.  
Cultures with reaction of  $+1$  are marked V for conidium-production.  
Cultures with reaction of  $+2$  are marked III for conidium-production.  
Cultures with reaction of  $+3$  are marked I for conidium-production.

#### Quality of Food

A study of table 2 at once shows that the quality of the food is a very important factor in the production of conidia. In solutions of carbohydrates no conidia are produced. Substrata which are very rich in proteins, as green bean pods, gluten, Duggar's agar and peptone, fall in column II for conidium-production. Those media which afford more of a balance of carbohydrate material and nitrogenous material, either organic or inor-



ganic, are the more favorable for conidium-production, as is seen by referring to columns IV and V. Materials having a low protein content, as polished rice and potato agar, show a low production of conidia, and the addition of a sugar solution, as levulose, does not stimulate conidium-production. A marked difference in conidium-production is shown when different sugars are added to a culture, either in an agar or as a solution to a filter-paper cone or cup. In oatmeal agar plus levulose a maximum number of conidia are produced, while oatmeal agar plus maltose in like percentage rates III in conidium-production. The addition of 1 percent of glycerin serves to reduce conidium-production on oatmeal agar from V to III and on potato agar from III to I.

#### Quantity of Food

The production of conidia is affected by the quantity of available food also. On oatmeal agar conidium-production is rated as V; on light oatmeal agar, which contains half as much oatmeal, conidium-production is rated IV; on water extract of oatmeal, conidium-production is rated II. Conidium-production on solution *e* falls in column IV, while that on solution *f* falls in column II. Solution *f* is different from solution *e* in that it calls for 10 percent levulose where *e* calls for 4 percent levulose. An interesting series of results are obtained from those cultures to which were added varying amounts of NaCl. Conidium-production is reduced on oat-agar cup cultures when the levulose solution in the capsule contains more than M/10 concentration of NaCl, and completely inhibited by more than 2 M concentration. Cultures on solution *e*, to which NaCl was added, are more sensitive. No reduction in conidium-production is produced by M/10 concentration of NaCl. M/1 concentration completely inhibits conidium-formation. Thus the filter-paper cone cultures are seen to be more sensitive to the concentration or toxic effect than are the oatmeal-agar cup cultures.

These results are at variance with those of Klebs on *Eurotium repens* (1896), wherein he demonstrated that conidium-production was most abundant on 15 percent solution of glucose. A reduction of this concentration caused a reduction in conidium-production. However, when he added NaCl to the solution in quantities sufficient to maintain an osmotic pressure equal to that of the 15 percent glucose solution, the maximum of conidium-production was maintained with a very small amount of glucose. In my cultures there is an evident toxicity with NaCl in concentrations above M/10. As this concentration is increased the toxic effect is more pronounced, until complete inhibition of conidium-formation is obtained. A reduction below this toxic minimum of an M/10 concentration produces no reduction of conidium-formation, which proves that the effect is not a concentration effect, but a specific toxicity.

The effect on conidium-production of a sudden removal of all or most of the food supply is demonstrated by the following experiment. Six filter-

paper cone cultures containing solution *g*, and six containing solution *h* were inoculated and allowed to grow for one month. At that time an examination was made to determine the degree of conidium-production. Under the low power of the microscope in specimens from cultures in solution *g*, the average number of conidia per field was thirty. In similar ones from solution *h*, the number averaged about twenty-five per field. Each of the filter-paper cones was then transferred by sterile forceps to large 200-cc. capsules containing sterile distilled water, and allowed to stand for thirty minutes. At the end of that time, of those from solution *g*, three were put in small capsules containing a fresh supply of solution *g*. The other three were put in small capsules containing sterile distilled water. The six cultures from solution *h* were handled in a like manner, three being put into fresh solution *h* and three into distilled water. These were again examined one month after these transfers were made. Those cultures which, after the washing in distilled water, were returned to fresh quantities of the nutrient solutions, were identical with the checks which had remained undisturbed in the nutrient solutions. There was no apparent increase in the amount of conidium-production during the second month. Those cultures which had been transferred to distilled water showed a very marked increase in conidium-production. Mounts from cultures remaining in solution *g* gave an average of 30 conidia per field of the microscope. Those from cultures which had been transferred from solution *g* to distilled water showed 100-150 per field in similar mounts. Mounts from cultures remaining in solution *h* showed an average of 25 conidia per field. Those which were transferred from *h* to distilled water showed from 100 to 200 conidia per field in similar mounts. It is seen from these results that those cultures which were grown in solution *h* produce, when transferred to distilled water, a greater percentage increase in conidium-production than do those from solution *g*.

The outstanding fact is that the transferring of culture growths from a rich solution to distilled water stimulates conidium-production to a very marked degree. This fact was first well demonstrated by the work of Klebs on *Saprolegnia mixta* in 1899. He showed that the nature of any specific development is conditioned by the environment to which the plant is subjected. Mention is made earlier in this paper of a number of other workers who have further demonstrated the validity of this principle. Pieters (1915) maintained that "the effect of a given environment may not become evident until the plant has been transferred to another environment." This is supported by the above-described experiment. Solution *h* equals solution *g* + 0.2 percent peptone. Vegetative growth in solution *g* is graded IV, while that in solution *h* is graded III. (See table 1). Although vegetative growth is less extensive in solution *h* than in solution *g*, the inverse is true of conidium-production when cultures in the two solutions are transferred to distilled water. The addition of peptone therefore

seems to be a stimulating factor which finds expression in an increased conidium-production after the culture has been transferred to distilled water, while it does not find such expression in those cultures which are kept in the rich solution.

TABLE 3. *Degree of Production of Stalked Bodies in Different Media*

O	I	II	III	IV	V
Pot. plugs	Green beans	Melilotus stems	Whole rice	Oat-agar cup and sol. <i>e</i> —MgSO <sub>4</sub>	Oat agar
Carrot plugs	Cornmeal	Rice paste	Wheat flour	Oat-agar cup and sol. <i>e</i> (Na for K)	Oat agar and 1% lev.
Pure wheat starch	Sol. <i>f</i>	Gluten	Sol. <i>e</i>	Plain agar cups and sol. <i>e</i>	Oat agar and 1% sucrose
Maltose 1%	Sol. <i>g</i> , Coons' sol.	Pot. agar	10% oat agar		Oat-agar cup and M/20 KH <sub>2</sub> PO <sub>4</sub>
Glucose 1%	Mod. of Coons' sol.	Oat agar and 1% glucose	Oat-agar cup and mod. Coons' sol.		Oat-agar cup and 1% lev. and M/20 KH <sub>2</sub> PO <sub>4</sub>
Levulose 1%	Prune-juice agar	Oat agar and 1% maltose	Oat-agar cup and 1% lev. and M/10 NaCl		Oat-agar cup and sol. <i>e</i>
Glucose 5%	Light oat agar	Pot. agar and 1% lev.			Oat-agar cup and 1% lev. and M/20 NaCl
Sol. <i>h</i>	Cornmeal agar and 1% lev.	Pot. agar and 1% sucrose Oat-agar cup and 1% maltose and M/20 KH <sub>2</sub> PO <sub>4</sub>			
Leonian's sol.	Light oat agar and 1% lev.				
Water extract of oatmeal	Plain agar cups and mod. of Coons' sol.	Oat-agar cup and Coons' sol.			
Beef-infusion agar	Sol. <i>e</i> and M/10 NaCl	Oat-agar cup and 1% lev. and M/5 NaCl			
Cornmeal agar Duggar's agar Duggar's agar and 0.2% peptone Leonian's agar Pot. agar and 1% glycerin Oat agar and 1% glycerin					

TABLE 3. *Continued.*

O	I	II	III	IV	V
Oat agar and 1% levulose and 1% gly- cerin					
Plain agar cup and Coons' sol.					
Oatmeal-agar cup and 1% lev. and M/2 NaCl					
Oat-agar cup and 1% lev. and M/1 NaCl					
Oat-agar cup and 1% lev. and 1.5 M NaCl					
Do. and 2 M NaCl					
Do. and 2.5 M NaCl					
Do. and 3 M NaCl					
Sol. <i>e</i> and M/5 NaCl					
Sol. <i>e</i> and M/2 NaCl					
Sol. <i>e</i> and M/1 NaCl					
Sol. <i>e</i> and 1.5 M NaCl					
Sol. <i>e</i> and 2 M NaCl					

## FACTORS INFLUENCING THE PRODUCTION OF STALKED BODIES

*Temperature*

The limits of temperature within which the growth of the stalked bodies occurs are rather narrow. None appear in cultures held consistently below 20° C. or in cultures maintained at temperatures above 25° C.. The optimum temperature ranges from 22°–24° C. Cultures kept constantly at 25° C. have not as large and vigorously growing bodies as those at 22°–24° C. As one approaches either the temperature minimum or temperature maximum for these bodies, they become fewer in number and smaller.

*Light*

Light, here as in the other phases of the life history of the plant, seems to be of rather small importance as an influencing factor. Cultures grown in the dark, however, show a greater number of vigorously growing bodies than do those grown in the light.

### *Oxygen Requirements*

Cultures from which a free circulation of air is excluded by sealing with paraffin fail to show any sign of the production of these stalked bodies.

### *Humidity*

Humidity of the culture chamber is not so much a factor in the number of the bodies developed as in the degree of development which they attain. On oatmeal-agar cultures in test tubes, which become comparatively dry after 50 to 60 days, the bodies seldom attain a height of more than 6 mm. In filter-paper cups containing this same agar, and a supply of moisture in the bottom of the capsule, the development of the bodies is more continued and extensive. In some of these cultures bodies are found which measure 10 mm. in height. This greater size is due, in part at least, to the maintenance of a plentiful supply of moisture.

### **Physico-chemical Factors**

The acidity or alkalinity of the medium very effectively limits the growth of these bodies. The reaction limits within which they may grow are wide enough, however, to include most common laboratory media.

In media with a reaction — 1, no bodies are produced.

In media with a reaction neutral, very few bodies are produced.

Media with a reaction + 1 are optimum.

In media with a reaction + 2, very few bodies are produced.

In media with a reaction + 3, no bodies are produced.

### **Quality of Food**

By far the most important factor in the production of these stalked bodies is the quality of the food materials. This is best brought out by a consideration of table 3. When compared with tables 1 and 2, it is seen that the number of different media which produce an optimum of growth of these bodies is very small in comparison with the number which are optimum for the other functions of the plant under consideration.

The outstanding fact to be observed in table 3 is that those media listed in column V all contain one essential substance, *viz.*, oatmeal agar. This oatmeal contains a specific combination of materials most favorable for the development of these bodies. The addition of other substances, such as levulose and salts, in some cases promotes and prolongs the vigor of growth to a small degree, but the effect is that of an increased or stimulated vigor of the plant as a whole rather than a specific effect on the production of these bodies. This was brought out by a series of cultures which were set up, using an M/20 solution of various salts, both with and without the presence of sugars. These salt solutions were added to oat-agar cup cul-

tures and grown for 3 to 6 months. The salts used were NaCl,  $K_2SO_4$ ,  $KH_2PO_4$ ,  $KNO_3$ ,  $Ca(NO_3)_2$ ,  $NaH_2PO_4$ , and  $Ca_3(PO_4)_2$ . These were added to cultures containing 1 percent levulose solution in one series and 1 percent sucrose in a second series. The bodies were produced in all the cultures. No difference could be seen in the growth on cultures to which was added 1 percent levulose alone, M/20  $KH_2PO_4$  alone, or a combination of the two. These did, however, show a more extensive development than those cultures on oatmeal alone. Of the other six salts used, none resulted in the production of quite so vigorous bodies as  $KH_2PO_4$ .  $K_2SO_4$  and  $Ca_3(PO_4)_2$  appeared least favorable for the production of the bodies, and afforded a less abundant production of the bodies than oatmeal agar alone.

The addition of 1 percent glycerin to the most favorable media, *viz.*, oatmeal-agar cups and 1 percent levulose, completely inhibits the growth of the bodies.

Levulose is the most favorable sugar for the stimulation of growth of the bodies. Sucrose is very nearly as good as levulose, but, considering the average over a very large number of cultures, it is found to be slightly less effective in inducing development of the bodies. Maltose is decidedly less favorable than either levulose or sucrose. Glucose is on a par with maltose and they both fall in column II, table 3, while levulose and sucrose fall in column V. This correlation between maltose and glucose, and sucrose and levulose, is quite to be expected, since maltose hydrolyzes into 2 molecules of glucose, and sucrose into 1 molecule of levulose and 1 of glucose. That this effect of the addition of levulose is purely a developmental stimulus, and not a formative one, is brought out by adding levulose to other media besides oatmeal agar, and by using it in solutions by itself. No bodies are produced on levulose solution, nor on any other pure sugar solutions. On cornmeal agar and levulose an occasional small body is produced. On potato agar a few bodies appear. The addition of levulose to the medium does not increase the number of bodies produced.

The nitrogenous content of the substratum used seems to be the most important factor in the production of the bodies. There is a specific protein content in oatmeal which makes it the most favorable of any substance used for the production of the bodies. Wheat flour (ordinary process), potatoes, and cornmeal are all rich in carbohydrates, but less rich in proteins than is oatmeal. On wheat flour, bodies are produced in smaller numbers, and these attain a lower degree of development, than do those on oatmeal. That the protein is the formative influence in the production of these bodies is demonstrated by the following experiment: The gluten was kneaded out of some flour from the same sample used for culture cups. This gluten was made as free from starch as possible. It was sterilized in filter-paper cups. At the same time, some pure wheat starch was placed in filter-paper cups and sterilized. These preparations were inoculated with the fungus. On the gluten cultures there was a sparse mycelial growth and

numerous stalked bodies were started. These did not develop well, however. In no instance did they attain a height of more than 1 mm. On the starch cultures there was an extensive mycelial growth, but no sign of the bodies. The replacing of the water in the gluten-cup cultures by 1 percent levulose solution did not induce any appreciable increase in the development in the bodies, although it did increase vegetative growth. Thus it appears that for the development of these bodies there must be present, along with a specific nitrogenous food content, some starch-bearing substance.

On synthetic solutions there is a marked variation in the production of these bodies. They are in no instance as favorable as is the oatmeal agar. Solution *e*, which falls in class III, is most favorable for the production of bodies of any solution used. Coons' solution falls in class I. Modification of Coons' solution falls in class I. Solution *e* employs as a nitrate  $\text{Ca}(\text{NO}_3)_2$ , while the modification of Coons' solution employs asparagin; otherwise the two solutions contain the same materials. Leonian's solution employs maltose and malt extract, both of which tend to inhibit body-formation, and in it no bodies at all are produced. The addition of malt extract to solution *e* reduces its rating for the production of bodies from III to I.

#### Quantity of Food

The influence of the quantity of food on the production of these bodies is best brought out by a comparison of the development on the standard oatmeal agar, and on that which contains one half the amount of oatmeal in the standard. In the standard, 60 grams of oatmeal are added to one liter. In the so-called light oatmeal agar, 30 grams of oatmeal are added to a liter. The production of bodies on the standard is V, while that on the light agar is I. The concentration of other added elements to the oatmeal agar may cause variations. The making of a 10 percent agar instead of the regular 1 percent concentration reduces body-production from V to III on an otherwise identical medium. The use of 10 percent levulose solution in preparing solution *f*, as compared with a 4 percent solution used in preparing solution *e*, reduces body-formation from III to I.

The production of these bodies is inhibited by a lower concentration of toxic substances than that affecting the other functions of the plant. No appreciable effect is seen on the addition of an M/20 solution of NaCl. The presence of an M/10 solution on NaCl, however, reduces body-production from V to III in an otherwise favorable medium. Increasing this concentration to M/2 of the NaCl completely inhibits the production of the bodies.

#### AN ALBINO MUTATION

*Brachysporium* is a genus of the Dematiaceae, and is characterized by dark brown, sometimes almost black, mycelium and conidia. The form under consideration, *Brachysporium trifolii*, was isolated from infected plants of white clover in October, 1919. All cultures were started from single



spores at that time. These have been kept going in culture as a pure line since that time, with frequent transfers to new media, with as many as 75 parallel cultures at one time on various media. When grown on various sorts of agar media, for example, the dark brown or blackish color is characteristic of all of them. On cornmeal agar the color is purplish black. On oatmeal agar a very similar color is developed. In liquid media, of different kinds, whether synthetic or composed of extracts of natural products, the growth is always densely black, tending to dark gray in the aërial portions; this is also its habit on the natural host.

In October, 1921, a little more than two years after first growing it on artificial media, a series was planted on oatmeal-agar cups. In one oatmeal-agar cup, of a set of sixty-seven cultures started at that time, there appeared a variation from the normal dark color. From the point of inoculation in the center of the agar cup, a sector grew out which lacked the usual color character. There was, in this sector, a growth of floccose hyphae, as is the habit for the fungus, and this grew out over the agar and down the sides of the cup in a manner quite like that of the normal type except that it was completely lacking in the dark color. The sector was completely colorless. After the usual period of time there appeared in the culture the stalked bodies which develop on this medium, and which, in the normal form, are densely black. They also appeared in the usual manner in the colorless area, but like the mycelium were absolutely devoid of the dark color. They were white to light flesh-colored instead.

Sub-cultures were made from the contrasting area of this culture, by transferring some of the white stalked bodies to petri dishes containing sterile agar. A number of these bodies, before being planted on the new agar dishes, were washed through three changes of sterile distilled water. This was done to remove any chance conidia from the blackened area which might have been carried over to the colorless sector in the handling of the culture. A pure albino growth was obtained from these planted bodies. These cultures were made on November 17, 1921, and this albino strain has remained unchanged in character up to the present time (May 1, 1922). Pedigreed cultures have been made, and the plant has been carried through seventeen non-sexual generations in culture. The conidia produced by this albino strain correspond in size and shape with those of the normal strain. Repeated single-spore isolations have been made, and these spores germinate readily, in a manner characteristic for the normal dark-colored form of the plant. These single-spore cultures remain constant for the character of albinism, as do the cultures made by isolating bits of mycelium.

The general cultural reactions of this albino form correspond in every way with that of the normal black form. The requirements for conidium-production, for production of bodies, and for vegetative growth are in general the same as are those for the normal type.

This sudden and striking change occurring once in a series of several

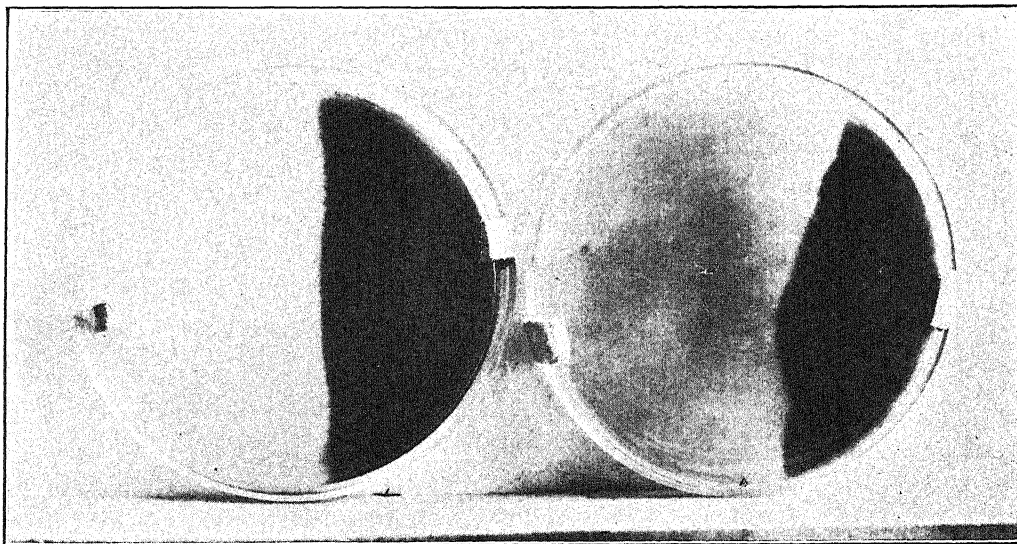
hundred cultures, and, showing so complete a transition in a single fundamental character of the organism, that of the dark color, will fall, it seems, under the category of a *bona fide* mutation.

A number of examples are cited in literature of mutations in fungi. These have in most cases had one general character in common, *viz.*, a dwarfing or reduction of development in the plant in some way. In some of these cases there is recorded simply a reduction in size or mass of growth of the fungus, while in other respects it retains the complete functioning of all its processes. Examples of such are mutants described by Schouten (1913) from *Rhizopus Oryzae*, and from *Phycomyces nitens*. Blakeslee (1920) describes what he calls "mutant A," from the hermaphroditic fungus *Mucor genevensis*. This mutant A differs from the parent form in having apparently lost its capacity to form zygospores. Blakeslee in the same article describes another type of mutant from the same species of *Mucor*. This second, "mutant X," is an example of a very decided dwarfing, so that all reproductive activity is lost, and only a reduced vegetation remains to represent the life history of the form. Schouten (1913) also describes one of this type: a mutation from *Dematium pullulans*, which not only lacks the capacity for reproduction, but also lacks the dark color of the normal type. These phenomena have all occurred in non-sexually propagated plants. They add, as Blakeslee points out, to the evidence that mutations are not restricted to processes involved in sexual reproduction.

Our case in hand, likewise, is an example of a mutation in a form which lacks any functional sexual process in its life history. This mutation is, however, somewhat different from those mentioned above. It is more clear-cut and striking, in that it is a change in one and only one factor, that of color. It does not involve a general reduction of growth or of some other life process. Usually such changes seem to have affected a group of factors. This appearance of an albino strain is a factor mutation. There simply seems to have appeared at one point in the formation of a new cell an irregularity, with the result that the new daughter cell did not carry the factor for color which is normal for this form. The new cell does, however, carry all the other factors for normal growth, asexual reproduction, etc., as is shown through a series of non-sexual generations studied in culture. This newly formed cell has not the factor for color, hence none of the subsequent growth from it can have the color, according to the indications of our knowledge and experience at the present time.

The contrasting appearance of this albino strain, which I have called strain A, with the original normal strain 1, from which A arose, is well shown by the photograph in text figure A. Although the mycelium of strain A does not show in the photograph in any way, the whole petri dish was covered by the growth of the two strains. These cultures were made by inoculating clear cornmeal-agar plates with the two strains, on opposite sides of the plate at the same time. Growth extends from the point of inoculation in a

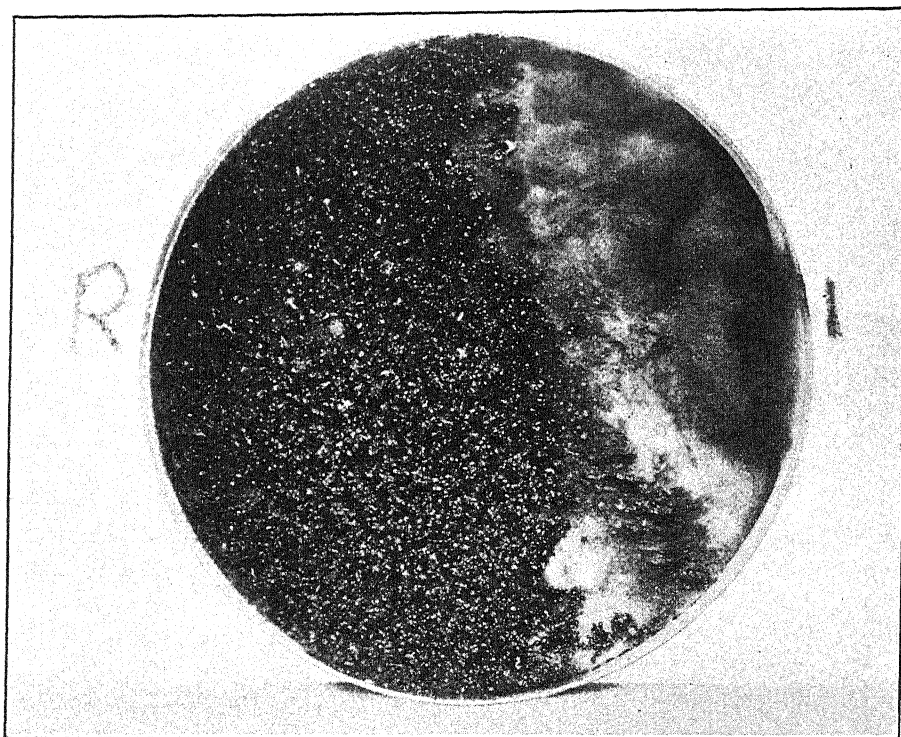
normal manner in both strains until they meet. A sharp line is formed by this meeting, and this line remains fixed. The black area does not encroach on the colorless area by later growth. The growth of strain *A* seems slightly more extensive than that of strain *I*, when averaging a large number of comparative cultures. There is slightly more aërial mycelium, however, in strain *I* than in strain *A*.



TEXT FIG. A. Cultures showing the contrast when strain *I* and strain *A* are planted at opposite sides of a plate of oatmeal agar.

Studies were made to determine, if possible, whether strains varying in some other way might be found in this species. The fact that this strain *I* had been grown in culture for more than two years, under a great variety of conditions, without having formed any mature fruit bodies, suggested the possible existence of a sexually different strain. Single-spore isolations were made from the original infected clover, which was collected at Washington, D. C., July, 1919. The spores of this material germinated in a typical manner after two days, and thirty-five single-spore isolations were made. These were designated as strains *B-Z*, and 2-11, respectively. Each of these thirty-five strains, each of which had come from a single spore, was plated on oatmeal-agar plates opposite to strain *I* and allowed to grow against it. These platings were repeated three times and seventy-five other promiscuous platings were made, using any two strains which might be taken. The result of this was that in no case was there any reaction toward reproductive activity induced by the meeting of the two strains. This shows that in those strains used there is no sexual differentiation. Each produces the usual growth of conidia and stalked bodies all over the substratum.

One very noticeable difference has, however, been brought to light in this series of cultures. This difference consists in the great excess of aërial mycelium formed by cultures of strain 1 as compared with cultures of the thirty-five newly isolated strains. Strain 1 forms a heavy, felty mat of mycelium all over the surface of the petri dish, sometimes as much as 5 mm. in depth. This mat tends to hide the stalked bodies from view in many cases.



TEXT FIG. B. Culture showing the contrast when strain 1 and strain *R* are grown, starting at opposite sides of a petri dish.

The bodies are, moreover, fewer in number than in the newly isolated strains. There are comparatively few normal upright conidiophores on this excessive growth of mycelium, and conidium-production as a whole is less abundant than on the newly isolated strains. In the more recently grown strains, an abundance of upright conidiophores cover the surface of the agar. These arise from a thin layer of mycelium over the surface of the agar. The felt-like growth of mycelium is of a gray color in some cultures, and is much lighter in color than the mycelium next to the agar.

Text figure B is a photograph of a six-weeks-old culture of strain *R* on one side of the dish, and strain 1 on the other side.

When strain *R* was planted on both sides of the dish, there was a uni-

form growth over the whole surface of the dish and no contrast could be seen. There was a uniform growth characteristic of strain 1 when it was planted on both sides of the dish. Any two of the recently isolated strains, picked at random from the 35, showed, when plated opposite each other, a uniform growth over the agar, and no difference appeared in the growth of the strains of the same age in culture.

Strain 1 had been in culture for 28 months at the time that the contrast cultures were made. Each of the other strains had been in culture for two months. Of the thirty-five newly isolated strains, no cultural differences could be detected between any of them. They are exceptionally uniform when considered through the series of cultures used as a whole. However, the effect on strain 1 of long-continued culture on rich substrata has been to cause a general reduction of the asexual reproductive activity and a concomitant increase in vegetative growth.

#### FURTHER STUDIES ON PATHOGENICITY

In the earlier report on this fungus (Bonar, 1920), some results of inoculation experiments were recorded. Those experiments had been made during the winter months of 1919-1920. The fungus was kept growing in pure culture, and in the autumn of 1920 further inoculation studies were begun. At this time a number of different species of *Trifolium*<sup>2</sup> were included in the experiments. The species used were: *T. incarnatum*, *T. pratense*, *T. hybridum*, *T. dubium*, *T. procumbens*, and *T. arvense*. When these had attained a vigorous growth in pots, they were placed in an inoculation chamber. This inoculation chamber consisted of a large glass-sided aquarium chamber with a loose-fitting glass plate cover. The humidity of this chamber was maintained at a high degree by keeping a small amount of water on the floor of the chamber. The pots of clover were placed in this chamber with their bases raised above the water level. Check plants grew luxuriantly in this chamber for three months' time, although they wilted severely when transferred to the benches in the greenhouse. Pots of the above-named species in a vigorously growing condition were placed in this chamber in January, 1921, and sprayed with a heavy suspension of conidia from a pure culture of *Brachysporium trifolii*. This strain of the fungus had been isolated from white clover in October, 1919, and had been grown on rich media, with frequent transfers, until the time when these inoculations were made. At the end of two weeks a very slight infection was apparent on the Landino variety of *T. repens* and on *T. dubium*. Final results were recorded at the end of three weeks as follows: *Trifolium pratense*, no infection; *T. incarnatum*, 1-3 percent of leaves and petioles infected; *T. hybridum*, 5 percent; *T. repens* (Dutch White), 5 percent; *T. repens latus* (Landino var.), 15 percent; *T. dubium*, 5-10 percent; *T. pro-*

<sup>2</sup>The writer is indebted to Dr. A. J. Pieters, of the U. S. Department of Agriculture, for seed of a number of species.



*cumbens*, 5 percent; *T. arvense*, no infection. The percentage of infection was estimated by the number of leaves and petioles that had been killed. The cause of this killing was proven by the presence of the typical growth of *Brachysporium* spores on the infected parts.

It appeared from these experiments that the fungus was only slightly pathogenic for *Trifolium repens* (Dutch White), which was used to demonstrate its pathogenicity in the experiments reported in the former paper. The Landino or *latus* variety of *Trifolium repens*, commonly grown in some parts of Italy, showed a greater degree of susceptibility to the fungus than did the commonly grown Dutch White variety of this country. A repetition of the experiment, using these two varieties, showed this to be true to an even more marked degree than was shown by the first trial. A very decided decrease in the pathogenicity of the fungus is shown when these results are compared with those of the experiments reported in the previous paper and carried on in 1919.

New cultures were then made from single spores isolated in January, 1922, from the original clover from Takoma Park, Maryland. This material, collected in July, 1919, had lain in herbarium pockets in the desk in the laboratory from October, 1919, until January, 1922. Inoculation experiments similar to those described above were made in February, 1922, using as inoculum spores from this newly isolated material, which had been cultivated on artificial media for six weeks. The species used in these last inoculation experiments were: *T. incarnatum*, *T. pratense*, *T. hybridum*, *T. repens* (Dutch White), *T. repens latus*, *T. dubium*, and *T. spumosum*. At the end of one week numerous yellowed leaves were to be seen on some pots. They were more evident on *T. spumosum* and *T. repens latus* than on any of the other species. At the end of two weeks from the time of inoculation the leaves of many plants had fallen to the ground, and examination of these dying parts with the microscope showed them to have a plentiful growth of *Brachysporium* conidiophores and conidia over their surfaces. At the end of the third week, records were made on the various species as follows:

*T. incarnatum*: 80 percent of the leaves and petioles dead. They had fallen as a brown mass, and examination showed them to bear a great abundance of *Brachysporium* conidia.

*T. pratense*: 25 percent of the leaves infected, and either fallen or partly so. Often only one leaflet of the three was killed. Conidia were abundant on the dead parts.

*T. hybridum*: 50 percent of the leaves and upper portions of petioles infected. Infection in many cases did not extend to the base of the petiole. Conidia were abundant on the dead parts.

*T. repens* (Dutch White): 20 percent infection. Complete collapse of infected parts. Conidia were abundant on the dead parts.

*T. repens latus*: 75 percent infection. Infection was more rapid than in other varieties. Conidia were abundant on the dead parts.

*T. dubium*: 60 percent infection. Some stalks were completely killed; others showed scattered dead leaves.

*T. spumosum*: Infection 100 percent; 90 percent of the leaves and petioles were killed. The leaves sometimes fell over while still green and healthy in appearance because of the collapse of the supporting petiole near the base. Conidia abundant.

Control plants were in good condition, showing no signs of any infection.

Plants of *T. repens* (Dutch White) and *T. repens latus*, which were sprayed with a suspension of *Brachysporium* spores and allowed to remain uncovered on the bench in the greenhouse showed no infection. A pot of *Trifolium dubium*, when sprayed with a suspension of conidia from strain A (albino) of *Brachysporium trifolii*, and kept under a bell jar, failed to show any infection.

It is evident from these experiments that the newly isolated strain is more vigorously pathogenic than the one that has been in culture continuously for twenty-eight months. This fungus is also shown to be more pathogenic, under the conditions of the inoculation chamber, to other species of *Trifolium* than it is to the Dutch White clover commonly distributed through our northern states. The most susceptible species is found to be *Trifolium spumosum*, while *T. incarnatum* and *T. repens latus* show a high degree of susceptibility.

A very high degree of humidity and a temperature of at least 75 to 80° F. are necessary conditions for infection. The following experiment demonstrates the effect of humidity on infection. A pot of *T. repens latus* was set on a board, sprayed with a suspension of *Brachysporium* spores, immediately covered by a bell jar, and allowed to stand for two weeks. A second similar pot was likewise sprayed with a spore suspension. This second pot, with its base protected by a glazed porcelain saucer, was set on a broad plate of somewhat greater diameter than the bell jar used for a cover. About a quarter of an inch of water was kept in the large plate. At the end of two weeks, the pot which rested on the board showed no infection, and the soil in the pot was dry. The plants showed definite signs of their need for water. In the second pot a very noticeable infection had taken place, and the air within the bell-jar chamber was highly saturated with moisture. The earth in which the plants were growing, however, had not received any addition of water. This demonstration of the high degree of humidity necessary for infection, coupled with the facts concerning the relation of temperature to spore germination given earlier in this paper, correlates readily with the results of the inoculation experiments. Given a high degree of saturation of the atmosphere and a comparatively high temperature, infection is possible on a number of species of the genus *Trifolium* when a recently isolated strain of the fungus is used for the experiment.

It becomes evident, from these results, that this fungus is not likely to be of considerable importance from an economic point of view. The con-



ditions ordinarily met in the open field will not fall within the range of those which are necessary for the infection of clover plants by *Brachysporium trifolii*. This opinion is corroborated by Dr. A. J. Pieters of the U. S. Department of Agriculture. He, using a culture of the fungus furnished him by the writer, failed to secure positive results in attempted inoculations in fields of white clover in the spring of 1921.

#### GENERAL DISCUSSION

The studies reported in this paper give a record of the life processes of *Brachysporium trifolii*, and, in more or less detail, of the factors conditioning the development or lack of development of the plant and its reproductive organs. The fungus presents, for the most part, no unusual morphological features as regards its vegetative growth or its asexual reproduction. The production, however, of large numbers of the peculiar stalk-like bodies on some substrata is worthy of more special consideration, since it was the elucidation of what these bodies represented that concerned the writer during the greater part of the experimentation. The question of the significance of these bodies in the life history of the organism is clouded by their failing to perform any evident function.

These bodies resemble sclerotia in the early stages of their development. The inner homogeneous medulla-like tissue, and the outer rind of thick-walled cells of a young body, correspond to the structure of a sclerotium. These can not, however, be sclerotia, since they, by further development, exceed the category of structures found in a sclerotium. De Bary (1887, p. 42) speaks of a "motley assemblage" of such "fungous bodies" which may later change by differential development. The formation of special interior areas, which are morphologically distinct from the surrounding tissue, argues in favor of their being the immature stage of some reproductive body. The method of development, by replacing the original tissue with a special growth of differentiated cells, precludes the possibility of its being the early development of a pycnidium. A number of workers have given us information on the structure and development of pycnidia (Reddick, 1911; Kempton, 1919). No record of any process of differentiation such as occurs in these bodies has been found in the literature on pycnidium-development. The cells of this interior area arise by differential growth from what was formerly homogeneous tissue, to form a special area of differential cells bounded in part at least by a distinct wall.

This development can, however, be compared with some of the recorded developments in the perithecia of certain Pyrenomycetes. Blackman and Welsford (1912) say that the ascogenous hyphae of *Polystigma rubrum* arise by differentiation from vegetative mycelium. They maintain that this occurs after the disintegration of a sexual apparatus consisting of an ascogonium and antheridium, which, according to them, are non-functional. Nienburg (1914), working on the same plant, shows by his figures a fertili-

zation process, but fails to show whether this has any relation to the formation of the ascogenous hyphae. Brooks (1910), in a study of *Gnomonia erythrostoma*, found, early in the development of the perithecium, what he considers to be an ascogonial apparatus. He did not, however, see any evidence that fertilization occurs. These cells disintegrate, and hence do not give rise to the ascogenous hyphae. They appear to arise late in the development of the perithecium after it is a well-formed homogeneous body of vegetative cells. Cayley (1921), in studies on the perithecia of *Nectria galligena*, makes a similar observation as to the origin of the ascogenous hyphae from apparently undifferentiated cells, rather than from any specialized sexual apparatus such as is known for *Ascobolus* or *Pyronema*. Reddick (1911) found that the pycnosclerotia of *Guignardia bidwellii*, which often remain sterile for a long time, may eventually develop into perithecia. These pycnosclerotia have certain central differentiated cells which show a much more pronounced staining reaction than the surrounding tissue. Noack (1905) definitely proved, by culture studies, that the bodies which had long been regarded as sclerotia in cultures of *Helminthosporium gramineum* were in fact immature perithecia of *Pleospora*, although Ravn (1901) had been able to secure only sterile growth after eighteen months in culture. From a consideration of these facts, I am led to the conclusion that the differential development in these stalked bodies in my cultures, represented in figures 9 and 10, Plate III, corresponds either to the early stages of the development of a perithecium, or to the development of an abortive body in part homologous with a perithecium. This disintegration continues until the protoplasm of these cells has completely disappeared (fig. 12). Although hundreds of these bodies have been carefully examined, no instance has been found of a more complete development of these perithecial primordia.

In search of another possible line of evidence on this subject, many young cultures, one to two weeks old, have been examined. It was thought that there might be some initial development on the agar which could be used to determine the origin of the development of the body as a whole. Brooks (1910) described the development of a coil-like structure as the initial of the perithecium of *Gnomonia erythrostoma*. My examinations, however, yielded only negative results.

Another line of argument which strongly supports the idea that these bodies are immature perithecia is afforded by a consideration of the physiological reactions of the plant as a whole. One of the principles most strongly brought out by Klebs (1899) is that the working limits of the general life conditions for a fungus are narrower for reproduction than for growth. He also goes on to show that the limits of conditions for the higher or sexual forms of reproduction are narrower than for asexual reproduction. He uses, as material for the support of this argument, data on *Sporodinia grandis*, *Saprolegnia mixta*, certain yeasts, and *Pleospora Sarcinulae*, as well as some others. This notion has been verified also for a large number of other

fungous forms. Other examples illustrating this same principle in perithecium-forming fungi are *Pleospora trichostoma* (Noack, 1905) and *Valsa leucostoma* (Leonian, 1921). A consideration of table 1 of the present paper shows that a large number of different substrata are favorable for at least a fair vegetative growth of this fungus. Table 2 shows that a considerably smaller number of these substrata are favorable for the production of conidia. Table 3 shows that a very much smaller number afford suitable food for the production of stalked bodies. The same relation holds for the other conditions of cultures as for the nature of the food stuffs mentioned above, e.g., temperature range, acidity or alkalinity, humidity, or the presence of some toxic substance. In this way the reactions of these stalked bodies are shown to correspond, in their general physiological behavior, to those known for the higher or sexual stage of reproduction of a considerable number of well studied forms. These facts, I think, support very strongly the argument for classing these stalked bodies as perithecia which for some reason do not attain a full development.

A number of cases are known in which complete development of Ascomycetes has been secured by employing some one special factor to fulfill what are otherwise incomplete conditions for development. Noack (1905) found that the sclerotia in cultures of *Helminthosporium gramineum* are stimulated to development into perithecia by exposing the cultures to a low temperature for a short time. Cultures of *Brachysporium trifolii* showing well developed, vigorously growing bodies have been placed in refrigerators at 0° and 10° C. and left for varying lengths of time up to one year. But neither after this treatment nor when they are again put under a higher temperature, is any more complete development found. Saito (1918) found that the addition of 4 to 10 percent NaCl to cultures of *Zygosaccharomyces major* stimulated the production of asci. When, however, NaCl was added to my cultures, there resulted an inhibition of the formation of the bodies, as is recorded earlier in this paper.

Cayley (1921) finds that the presence in the culture medium of a small percentage of glycerin is necessary for the formation of the ascus stage of *Nectria gallegina*. The addition of 0.5 to 1 percent glycerin to the cultures in my study absolutely inhibited all production of the bodies. Leonian (1921) found that the addition of sugars to a rich oatmeal agar induced the formation of perithecia in *Valsa leucostoma*. In *Brachysporium*, however, the addition of levulose, although it induces a more vigorous and prolonged general development of the plant, does not produce a greater degree of differentiation in the stalked bodies. Sucrose produces a similar effect, although the stimulation is slightly less pronounced than when levulose is used.

The existence, within a single fungous species, of complementary strains, the presence of both of which is necessary for sexual development, has been demonstrated for a wide variety of forms. Blakeslee (1904) designated

these as "plus" and "minus" strains when he first discovered them in the Mucorales. Edgerton (1914) and Dodge (1920) report the existence of complementary strains in the Ascomycetes. Many cultures were therefore made, as described earlier in this paper, to test the possibility of securing a reproductive reaction between two different strains. None such was secured, nor did any indication appear of any tendency toward such a reaction. Blakeslee (1906) says, when speaking of algae and fungi:

The absence of sexual reproduction may be due: (1) to constitutional sterility; (2) to conditions of growth unfavorable to the production of sexual organs; or (3) to the fact that the form is heterothallic and that the thalli of both sexes have not been brought together.

The first category hardly covers the material under consideration, since a very plentiful development of the bodies to a fairly advanced stage is obtained. The large number of variations of conditions employed in this culture work tends to throw the weight of evidence away from the second possibility. All of my experiments to show that the third supposition might apply to the fungus under study have, likewise, given negative results.

After considering all the facts concerning the nature of these bodies which develop in cultures, we are led to the conclusion that they represent a degenerate stage of what was once a perithecial form of reproduction.

A very interesting example of the attenuation of the virulence of a fungus through long-continued culture on artificial media is afforded by this organism. Krakover (1917) says:

It is commonly asserted that parasitic fungi lose their virulence when kept in culture for a long time, but exact experimental evidence is not at hand.

He gave an example of attenuation in *Macrosporium sarcinaeforme* from red clover. The attenuation of the virulence of *Brachysporium trifolii* after continued culture, as discussed earlier in this paper, is coincident with an increase in vegetative growth at the expense of reproductive activity. There seems to have occurred, in this long-cultivated strain 1, a certain reduction in the scale of its life activities. There has been a loss of virulence and of some of its reproductive activity, both of which are more specialized characteristics than mere vegetative growth, which has been increased. This is shown to be true when these later cultures of strain 1 are compared with its original virulence and with its original habit of growth in culture. It is also shown to be true when compared with the thirty-five recently isolated strains from the original collection of material, even after the latter has been dried for a long time. This variation is not to be placed in the same category with a mutation nor confused with that idea. An example of a mutational change is afforded by strain A. This mutation represents a clear and distinct change of a definite nature, comprising all the morphological structures of the fungus, which occurs suddenly and remains distinct without any apparent other change or changed relations to any of the regular life processes of the organism.

## SUMMARY

1. *Brachysporium trifolii* Kauff. has been studied with regard to its physiological reactions and its complete life history both in culture and on its native host.

2. Very numerous slender black bodies are produced in certain cultures. These bodies are at first composed of homogeneous pseudoparenchymatous tissue. Later growth shows a differentiation of definite interior areas in this homogeneous tissue. These interior areas resemble the early development within a young perithecium. At a certain stage, a disintegration of the protoplasm sets in within the cells of this inner area, and the result is a sterile tissue which undergoes no further development.

3. From among a large number of food materials used, heavy oatmeal agar is the most favorable for the production of the stalked bodies.

4. The development of the mycelium, conidia, or bodies is to a very large degree conditioned by the environment under which the fungus is grown. The organism is found to have a wider range of conditions suitable for vegetative growth than for asexual reproduction. The conditions for asexual reproduction are, likewise, much more general than those for the production of the stalked bodies. This relation of the different reactions corresponds with the reactions of fungi in general.

5. Considered from the standpoint of morphology, method of development, and general physiological reactions, the stalked bodies seem to be immature perithecia, or, in part at least, homologous with perithecia.

6. A distinct albino mutation has been found once in a long-cultivated pure line of the normal dark-colored form.

7. Long-continued growth of the fungus in culture causes an attenuation of its virulence.

8. Different species of *Trifolium* show varying degrees of susceptibility to attack by *Brachysporium trifolii*, under the conditions of the inoculation chamber.

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## DESCRIPTION OF PLATES

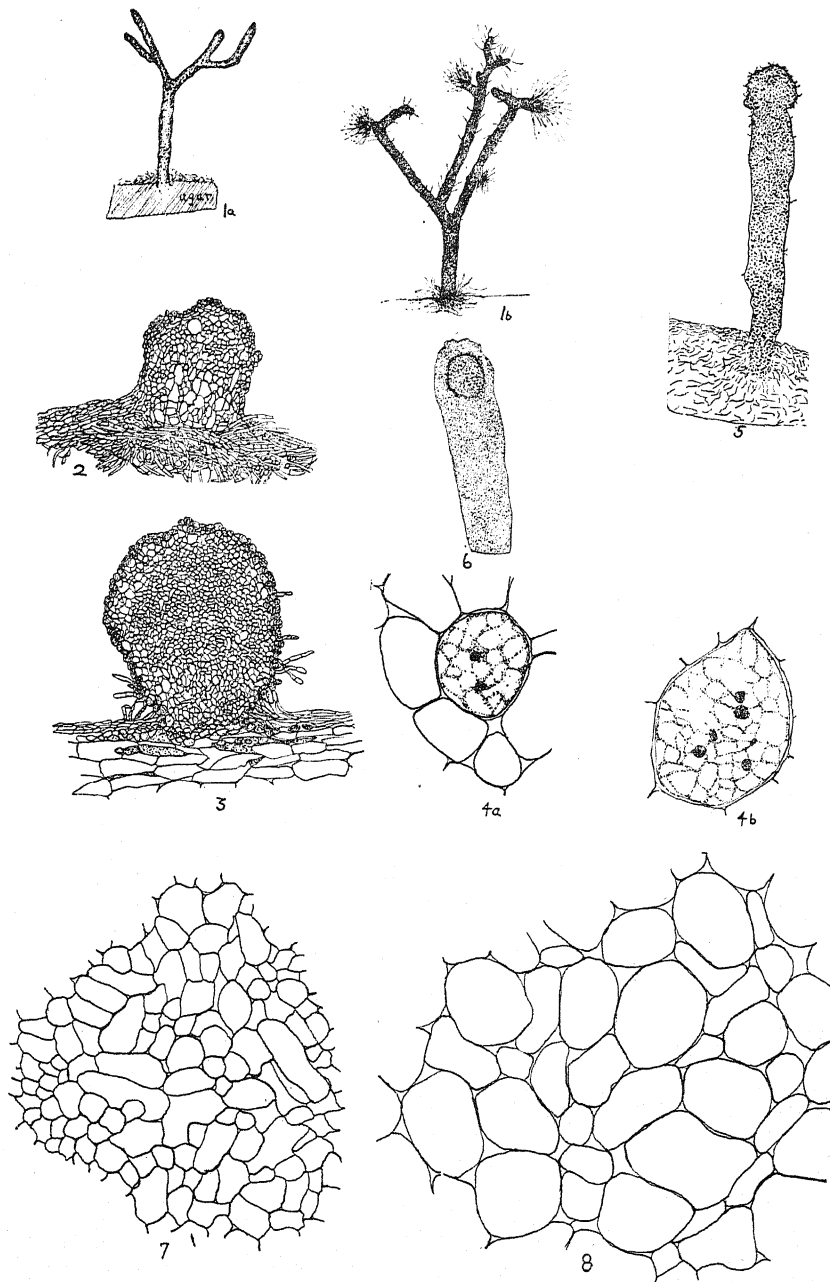
## PLATE II

- FIGS. 1 *a, b*. *a*, one of the stalked bodies 45 days old; *b*, one 60 days old.  $\times 5$ .
- FIG. 2. Longitudinal section of a young body, showing its relation to the agar from which it grew.  $\times 125$ .
- FIG. 3. Longitudinal section of a young body, showing its origin on a stem of *Melilotus* on which it was grown.  $\times 125$ .
- FIGS. 4 *a, b*. Cells from the young bodies shown in figures 2 and 3.  $\times 1425$ .
- FIG. 5. Longitudinal section through the length of one of the bodies 25 days old.  $\times 32$ .
- FIG. 6. Longitudinal section through the tip of a body 60 days old, showing the differentiated area within.  $\times 32$ .
- FIG. 7. Cells from the undifferentiated portion of a 60-day-old body.  $\times 1260$ .
- FIG. 8. Cells from the inner differentiated area of the same body shown in figure 7.  $\times 1260$ .

## PLATE III

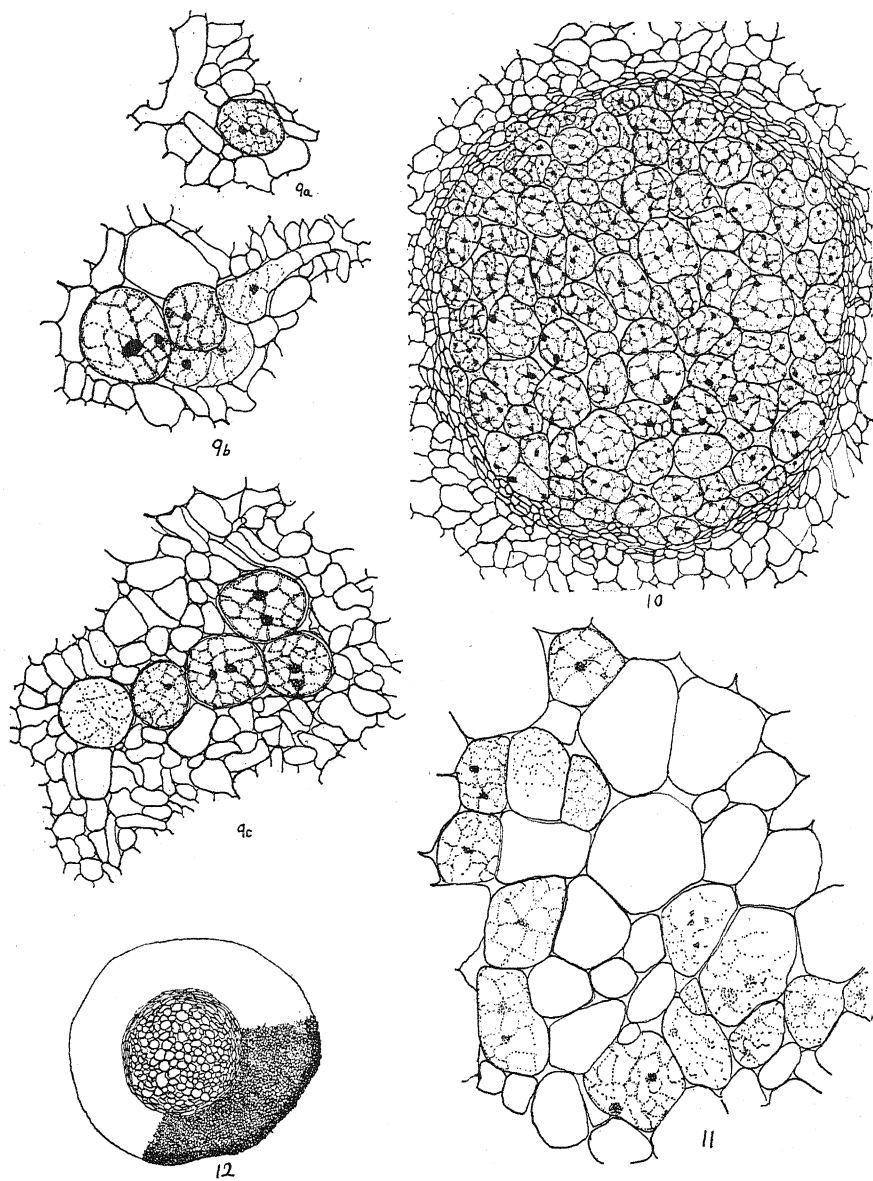
- FIGS. 9 *a, b, c*. Stages in the differentiation and development of the inner areas from the ordinary body cells.  $\times 1260$ .
- FIG. 10. Further development of a differentiated area and the formation of the limiting wall.  $\times 1260$ .
- FIG. 11. Disintegration of the protoplasm of the cells in the inner area, after the greatest recorded development has been attained.  $\times 1425$ .
- FIG. 12. Cross section through one of the bodies, showing the different areas after there has been a complete loss of all the protoplasm from all the cells.  $\times 125$ .





BONAR: BIOLOGY OF BRACHYSPORIUM TRIFOLII





BONAR: BIOLOGY OF BRACHYSPORIUM TRIFOLII



## HYGROCHASTIC MOVEMENTS IN FLORAL BRACTS OF AMMOBIUM, ACROCLINIUM, RHODANTHE, AND HELICHRYSUM

J. C. TH. UPHOF

(Received for publication May 28, 1923)

Organs of a certain number of plants undergo movements upon becoming either wet or dry which are called by Ascherson (1) *hygrochastic* movements. Well-known examples are *Anastatica hierochuntica* and *Asteriscus pygmaeus* (5), both species being well known desert plants of northern Africa and Palestine. They curl up on becoming dry and spread out again after being moistened. In the former the small twigs are the cause of the movements, whereas in the latter plants the bracts surrounding the inflorescence change location. This work has been sufficiently explained by Steinbrinck and Schinz (3).

The writer (4) made extensive investigations on the physiological anatomy of xerophytic Selaginellas found in various deserts of North and South America, Africa, and Asia, especially of xerophytic species belonging to the Heterophyllum group, including *Selaginella Pringlei*, *S. lepidophylla*, *S. pilifera*, and other species.

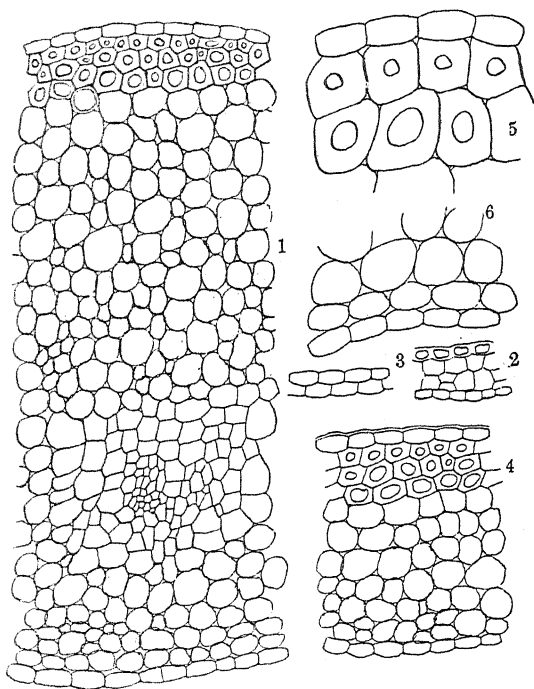
The anatomical construction of these forms shows a rather thin-walled epidermis, hypodermis, and cortex on the upper surface of the stem, and a thick-walled epidermis, hypodermis, and cortex on the lower surface. During drought the thin-walled tissue loses more water than the thick-walled tissue, in consequence of which the plant curls up. It absorbs more water when moistened, and spreads out. The hygrochastic movements are mechanical and can be observed in dead as well as in living plants.

Exactly the reverse of the movements above mentioned may be noticed in the bracts surrounding the flower heads of *Acroclinium roseum* Hook., *Helichrysum bracteatum* Andr., *Ammobium alatum* R. Br., and *Rhodanthe Manglesii* Lindl. They close when moistened, thus making a shelter for the flowers during rain and protecting the pollen and other structures; they open again upon becoming dry. These movements are far more rapid than in *Selaginella*, *Anastatica*, or *Asteriscus*; in fact, they are almost as rapid as the movements of the leaves of *Mimosa pudica* when touched.

The main features of the mechanical tissues in the various species studied by the author were essentially the same.

Differently located bracts, namely, those of the inside and those toward the outside of the inflorescence, were arranged for cutting with the Spencer rotary microtome, cut at a thickness of 5 microns and stained in Delafield's haematoxylin.

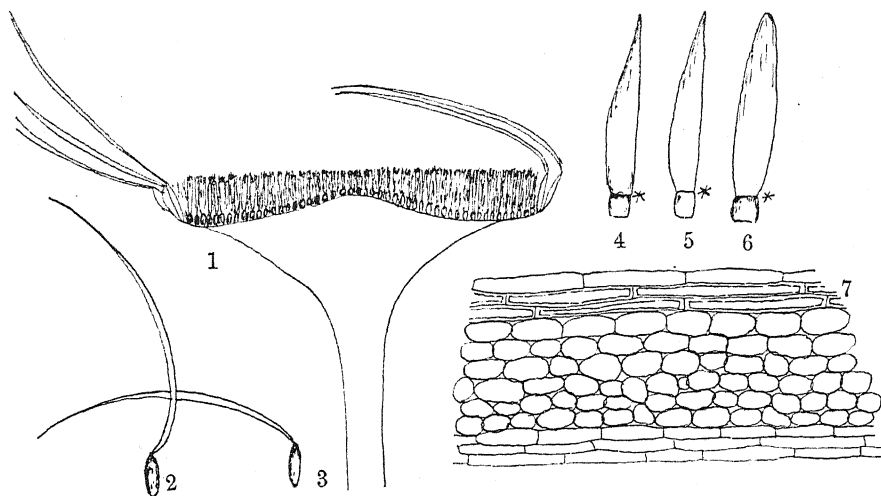
The greatest differences in anatomical structure are at once evident. Toward the base of the bract (text fig. A, 1), which is composed of several layers of cells, is located usually one fibro-vascular bundle.



TEXT FIG. A. 1, Cross section of lower part of bract, toward the middle, of *Acroclinium roseum*. 2 and 4, Cross sections of the same toward the sides. 3, Cross section through upper part of bract. 5, Upper part of fig. 1 enlarged. 6, Lower part of fig. 1 enlarged.

The difference between the upper and lower surfaces in cross section is very conspicuous (text fig. A, 1, 4, 5, 6.). The cells of the upper epidermis are small and are usually, though not always, provided with a relatively thick cuticle. The epidermis, especially toward the middle, is followed by a tissue of three or four layers of thick-walled cells. These cells have small lumina, and between them are only small intercellular spaces. The thick-walled tissue is followed by an extensive parenchyma with larger intercellular spaces and thin-walled cells. Imbedded in this tissue we find the small fibro-vascular bundle, which apparently takes no part in the hygrochastic movements of the bracts. The thin-walled tissue extends to the lower epidermis, which is similar in appearance to the upper epidermis, with the exception that there is no noticeable cuticle. In cross section the walls are almost round, in longitudinal section they are rectangular (text fig. B, 7).

It is at once evident that these tissues play an important part in the hygrochastic movements. It is naturally suggested that a differentiation in water-absorbing power between the upper and lower surfaces is the cause of the movements of the floral bracts, and this is actually the case. The lower thin-walled parenchyma absorbs more water than the parenchyma of the upper surface; this causes a curling up of the bracts toward the inner side, thus protecting the flowers against moisture.



TEXT FIG. B. 1, Section of flower head, the left when dry, the right when wet. 2, Situation of bract when dry. 3, Situation of bract when wet. 4, Bract of *Ammobium alatum*. 5, Bract of *Rhodanthe Manglesii*. 6, Bract of *Acroclinium roseum*. The major movement occurs at the point marked \*. 7, Longitudinal section of bract of *Acroclinium roseum*.

The differences of expansion of the various tissues due to unequal absorption can easily be measured and compared in longitudinal section, as is shown by table I.

TABLE I. Increase in Length of Upper and Lower Surfaces of Bracts due to Absorption, as Compared with Dry Tissue

Name of Plant	Location of Part of Bract Measured	Length in Microns of both Tissues when Wet	Microns of Thick-walled Tissue when Dry	Microns of Thin-walled Tissue when Dry
<i>Ammobium alatum</i> .....	at base.....	1000	991	972
	2 mm. from base....	876	853	829
<i>Acroclinium roseum</i> .....	at base.....	1230	1206	1132
	2 mm. from base....	1040	1024	998
<i>Helichrysum bracteatum</i> ....	at base.....	871	853	835
	2 mm. from base....	1136	1121	1102
<i>Rhodanthe Manglesii</i> .....	at base.....	645	632	625
	2 mm. from base....	875	869	862



Upon examining the upper half of the bracts of the various species, it is at once clear that the tissues are here much thinner-walled. Toward the middle of the bract they are composed of four layers; toward the apex, of but two layers of cells (text fig. A, 2 and 3). It is noticeable that the cells are here everywhere thin-walled; also that the cuticle is very slightly developed.

When moistened it can be easily observed that the part played by these portions of the bract in the actual causation of the movement is very little, indeed almost nothing, as is to be expected from their anatomical construction, whereas the lower half of the bract (text fig. A, 1), which contains thick-walled cells near its upper surface, performs the actual movements. This part very distinctly and very quickly turns the upper half of the bract toward the inside of the inflorescence. The apex of the bracts of *Acroclinium roseum* and *Helichrysum bracteatum* pass the center and form a small angle, whereas in *Rhodanthe* the bracts form a kind of cap over the flower-head, and stand at a larger angle in relation to their base. When, for example, a bract is removed from *Acroclinium*, it takes the position shown in text figure B, 2, when dry, but when moistened it very quickly moves so as to take on the position shown in text figure B, 3. These experiments can be repeated several times within an hour, provided that the tissues are allowed to dry soon after having been moistened.

Table 2 gives measurements of the upper and lower surface tissues in the upper halves of the flower bracts before and after being moistened.

TABLE 2. Increase in Length of Upper and Lower Surfaces of Bracts Due to Absorption, as Compared with Dry Tissue

Name of Plant	Location of Part of Bract Measured	Length of both Tissues in Microns when Dry	Length in Microns of Upper Surface when Dry	Length in Microns of Lower Surface when Dry
<i>Ammobium alatum</i> .....	1 mm. from apex.....	960	952	951
	3 mm. from apex....	1120	1109	1109
<i>Acroclinium roseum</i> .....	1 mm. from apex.....	1074	1061	1060
	3 mm. from apex....	844	831	830
<i>Helichrysum bracteatum</i> ..	1 mm. from apex.....	1024	999	998
	3 mm. from apex....	1186	1172	1171
<i>Rhodanthe Manglesii</i> .....	1 mm. from apex.....	874	862	862
	3 mm. from apex....	984	961	961

Table 2 shows that there is no marked difference in absorbing power between the upper and the lower surfaces. When wet, the two surfaces expand normally in the same proportion.

The movement of the bracts begins when the flowers are well developed. Before this time the cells of the bracts contain protoplasm and a considerable amount of moisture, and in young stages the walls of the upper surface cells have no secondary layers. Later, however, the walls increase in thickness, the protoplasm disappears, and the bracts spread out upon becoming dry, thus exposing the flowers.

The movements of the bracts are now entirely physical; when moistened by rain they close the inflorescence, and when dry they again bend outward. The same movements are observed when the bracts have been in a thermostat under a temperature of 120° C.; they also occur when the bracts have been submerged in 100 percent alcohol, treated with xylol, and afterward dried again. There is no doubt, therefore, that the hygrochastic movements are not characteristic of the living protoplasm.

#### SUMMARY

1. The anatomical construction of the bracts of *Ammobium*, *Acroclinium*, *Rhodanthe*, and *Helichrysum* is entirely the reverse of that characterizing xerophytic *Selaginellas*, *Anastatica*, and *Asteriscus*. The hygrochastic movements are consequently in the opposite direction.

2. In the four *Compositae* first named, the mechanical tissue is thick-walled near the upper surface and thin-walled toward the lower surface, which difference causes the bracts to curl toward the middle when moistened.

3. The cause of the movements is physical rather than biophysical, since the cells are deprived of living protoplasm.

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# THE INFLUENCE OF VITAMINS UPON THE DEVELOPMENT OF YEASTS AND MOLDS

## (CONTRIBUTION TO THE BIOS PROBLEM)

W. LEPESCHKIN

(Received for publication June 5, 1923)

Pasteur in 1871 indicated that the development of yeasts may depend upon the original number of cells brought into the culture solution. Wildiers (1901) found that yeasts can not develop in a solution containing ammonium salts as the only source of nitrogen. But the development becomes possible if a quantity of yeast extract is added to the solution. Wildiers called the unknown substance present in the yeast extract and necessary for the development of yeasts by the name "bios."

The results of Wildiers were later confirmed by some authors, but the majority of them considered the absence of yeast growth as a consequence of insufficient food in the culture solution.

Lately, Pringsheim (1916) has concluded that the development of a yeast, if it is brought into the culture solution in great quantity, becomes possible in consequence of an exudation of food substances from the dead cells. If the quantity of yeast supplied is very small, this help disappears. According to the experiments of Pringsheim, a repeated sowing of yeast cells in fresh culture solution containing mineral nitrogen resulted in an accommodation of the yeast, so that single cells became able to grow in this solution.

Lindner (1920) gives another explanation of the failure of single cells to develop. This author points out that the growth of yeast decreases with the formation of fat or alcohol in the cells and that the presence of oxygen and poor nitrogen nutrition favor this transformation. If the yeast is present in great quantity in a culture solution containing mineral nitrogen, the competition of neighboring cells results in an insufficiency of oxygen and growth becomes possible. On the contrary, in the case of isolated cells the excess of oxygen brings about the transformation of fat or alcohol and hinders the growth of the yeast.

The discovery of so-called completion-substances or vitamins permits us to regard the bios problem from a new point of view. Indeed, the yeast is found to contain vitamins which exercise an accelerating effect upon different physiological functions of animals. Therefore, it is now interesting to test whether these vitamins are necessary for the life and growth of the yeast itself.

I will briefly describe in this paper some experiments which were made for this purpose in my laboratory by Mr. A. Votschal in Kasán (1916). The experiments were made with the pure culture of *Sacchomyces cerevisiae* I Hansen.

If the "Tröpfchenkultur" of Lindner was made with the above-named yeast in such a manner that a single cell was present in each drop of the culture solution containing ammonium sulphate as the source of nitrogen, no development of the yeast was observed. On the contrary, if the culture liquid contained peptone, the development was excellent. The result was the same if the single cells were transferred from damp chambers to bottles filled with the culture solutions. Yeast extract could be substituted for peptone and the development was also excellent. If the amount of yeast originally present was great, a development was observed in all solutions. Therefore, the suggestion of Lindner did not prove correct; on the bottom of the bottles filled with the sterilized solution the presence of an excess of oxygen could scarcely be admitted.

In order to make clear whether the single yeast cells were unable to grow only in consequence of an insufficiency or absence of vitamin, the same experiment was carried out except that a very small quantity of vitamin B was added to the culture solution. This vitamin was obtained from yeast according to the instructions of Func. An addition of 0.001 percent of crystallized vitamin (melting point  $233^{\circ}$ , molecular weight 511) to the culture solution containing ammonium sulphate as the single source of nitrogen was sufficient to make possible the development of the yeast. Peptone, which produced, as mentioned, an analogous effect, also contains, as our experiments showed, vitamin B.

Bachmann (1919) has recently shown that the yeast growing at the surface of the liquid is less dependent on vitamin. In any event it is evident that the yeast, being in a state of good development, is able to synthesize the vitamin. If single cells of bottom yeast or of *Saccharomyces cerevisiae* I were not able to begin growth without adding supplementary vitamin, this fact may be explained by the suggestion that the vitamin diffuses too readily into the surrounding solution, as is the case with the co-enzyme (Euler and Karlson, 1919).

On the other hand, vitamin, when present in the culture solution in a sufficient quantity, increased not only the rate of fermentation of the yeast, but also very strongly its rate of growth. This was demonstrated by the following experiment.

The yeast to be tested was distributed very homogeneously in the culture solution (by means of shaking), so that each drop of the liquid placed on the under side of a cover glass in a damp chamber contained 2 or 3 cells. Then the development of the yeast was observed under the microscope, and the number of cells was determined from time to time. The standard solution contained 10 percent glucose, 0.1 percent asparagin, 0.1 percent  $MgSO_4$ ,

and 0.2 percent  $\text{KH}_2\text{PO}_4$ . The quantities of vitamin added to the solutions were, respectively, 0.01 percent, 0.001 percent, and 0.0001 percent. In order to weigh the small quantity of vitamin a micro-weighing machine (Nerst's, 1913) was used. The following numbers express percentage of overgrowth of the yeast in proportion to the original number of cells. The numbers average (from four experiments):

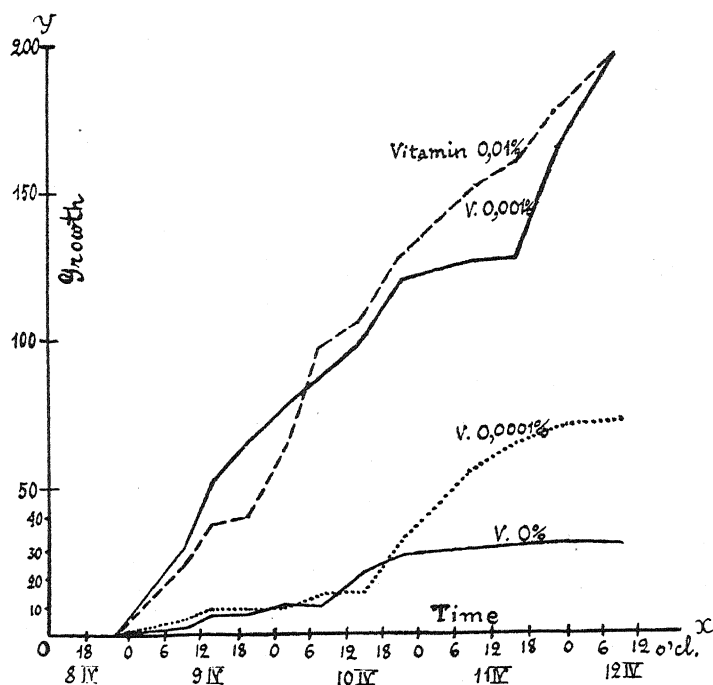
Dates	Quantities of Vitamin Added			
	0	0.01%	0.001%	0.0001%
April 8, 10:30 A.M.	Percentage of Overgrowth			
April 9, 9:30 A.M.	1.7	25.0	29.4	4.2
April 9, 2:30 P.M.	8.4	38.4	52.9	9.7
April 9, 4 P.M.	8.4	41.6	67.6	9.7
April 10, 3 A.M.	10.2	67.3	79.4	9.7
April 10, 8:30 A.M.	10.2	94.2	88.2	12.5
April 10, 3 P.M.	22.0	105.7	94.1	12.5
April 10, 11:30 P.M.	25.4	128.9	120.6	31.8
April 11, 8 A.M.	27.1	150.0	126.4	55.0
April 11, 4:30 P.M.	27.1	159.6	126.4	65.0
April 11, 12 midnight	27.9	176.9	167.6	71.0
April 12, 10:30 A.M.	27.9	196.1	197.0	75.0

If we plot the obtained percentages of overgrowth on the  $y$  axis and the times of observations on the  $x$  axis of a coordinate system, we obtain the growth curves shown in text figure 1. These curves indicate that no important difference was observed between the effect produced on the growth of the yeast by 0.01 percent vitamin and that produced by 0.001 percent vitamin. On the contrary, 0.0001 percent vitamin produced a much weaker effect. It appears, therefore, that the yeast requires only a small quantity of vitamin, and that it is useless to increase this quantity.

The effect of the vitamin upon the growth of the yeast can, of course, be remarkable only in the case of an excess of food, and, if the culture lasts for many days, no difference is observed between the quantities of yeast in all solutions.

Concerning the necessity of vitamins for the growth of molds, Currie (1917) and Willaman (1920) found *Aspergillus niger* to require no addition of any completion substance to the culture solutions. But *Sclerotinia cinerea* could not grow without the addition of fruit juices, extract of yeast, etc., to the culture solutions. The experiments were made, in our laboratory, with *Penicillium glaucum*. The effect produced by the vitamin was found to be like that produced upon the yeast; it was observed only in the first days of the culture when the mold had an excess of food, and no difference was observed if the culture lasted many days. An exceedingly great difference in the first phases of growth could be observed directly under the microscope, if the spores of the mold germinated in a damp chamber.

The entire length of the hyphae of the mold increased, in our experiments, 200 percent without vitamin, and 3483 percent if 0.01 percent vitamin was



TEXT FIG. 1. Graph showing the effect of vitamin on the growth of yeast.

added to the culture solution ( $2\frac{1}{2}$  days at room temperature). The standard solution contained 50 g. of saccharose, 2 g.  $\text{KH}_2\text{PO}_4$ , 1 g.  $\text{MgSO}_4$ , 2.6 g.  $(\text{NH}_4)\text{NO}_3$ , and 0.0046 g.  $\text{FeSO}_4$  in 1000 g. water.

Concerning the cause of the effect of the vitamin, it may probably be either catalytic or similar to the effect of the co-enzyme in the fermentation process, for growth may probably also be a catalytic process.

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# CONCERNING THE CHARACTERS OF CERTAIN FUNGI AS EXHIBITED BY THEIR GROWTH IN THE PRESENCE OF OTHER FUNGI

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(Received for publication June 23, 1923)

## INTRODUCTION

In common with all other organisms, fungi are modified by the changes in their environment. The presence of other living forms constitutes a part of the environment, and consequently these affect the morphology and physiology of the fungi; thus, growth is usually checked when two or more fungi are contiguous. Such inhibition may be mutual, or the growth of one individual may be inhibited more than that of the other. The antagonistic action of one fungus toward another may be due to a variety of causes, and results in numerous modifications of the organisms involved. The study of growth changes so induced is the purpose of this paper.

## METHODS

In the routine work of determining types of inhibition, the various organisms used were grown on cornmeal agar in petri plates. Effort was made always to have the conditions as nearly uniform as possible. Variations were made from this routine procedure in order to observe the growth phenomena under different conditions.

*A.* The depth of the medium was varied by permitting the agar to harden in the plates while the plates were tilted. Colonies were then planted in the shallow and deep regions of the agar.

*B.* The medium was varied as to the amount and kinds of nutrients present, employing washed agar, plain agar, cornmeal agar, dextrose agar, and Brazil-nut agar.

*C.* The time element was varied by making inoculations at periods ranging from 24 to 129 hours from the time of the initial transfer.

*D.* The amount of inoculum was varied.

In noting morphological changes, direct observations were made through the microscope. One other method that proved very successful was as follows: Thin layers of agar were carefully poured on cooled sterile cover slips, where the agar hardened immediately. Slips thus prepared were inoculated with two fungi that were to be placed under observation. The inoculations were made so that the developing colonies would not be more than 1 to 2 cm. from each other. As soon as development was well started, the cover slips were mounted on the stage of the microscope and the mi-



croscope was placed in position on the stand of a Bausch and Lomb photo-micro-projection apparatus. In this manner the fungous hyphae were enormously magnified, and even minute changes in appearance and the manner and quality of the change could be quickly noted. Observations were made at short intervals, and the light was not kept on longer than necessary to make observations lest the illumination modify the growth in some manner.

Aseptic seedlings were secured and placed in rag-dolls in the manner suggested by F. L. Stevens (41). The dolls were divided into four lots, the lots being subjected to the following treatments: (1) Several loopfuls of *Helminthosporium* spores were rubbed on the outside of the cloth surrounding the seedlings, and the whole roll was then dipped into a broth suspension of organism no. 45. (2) Treated as lot one, except that the rolls were not dipped into the broth suspension. (3) Rolls containing the seedlings were dipped into the broth suspension but were not inoculated with the spores of *Helminthosporium*. (4) Rolls were placed in their tubes without being inoculated with the spores of *Helminthosporium* or dipped into a broth suspension of organism no. 45. Lots 3 and 4 served as controls.

Pot cultures were secured as follows: Five-inch pots were filled with earth. Cones, one inch in diameter at the top and three and one half inches long, made of thin paper, were filled with earth which had been thoroughly moistened with a broth culture of organism no. 45. These were sunk in the center of the pots. Inside these cones were placed a number of flax seeds that had been so treated as to render them aseptic. The soil outside the cones was inoculated with the spores of *Fusarium lini*. In the control pots the soil in the cones was moistened with sterile broth only.

The staling products of *Penicillium* growth were secured in the following manner: Sterile orange juice was inoculated with *Penicillium italicum* and kept at room temperature for six weeks. At the end of this time the juice was filtered through a Reichel filter. The filtrate was placed in sterile petri dishes and permitted to stand for several weeks in a sterile desiccator over calcium chlorid. In this time the filtrate was considerably concentrated. The liquid was injected into the oranges with a Leur syringe.

#### ACCESSION LIST OF ORGANISMS

The following organisms are those made use of in my experiments. Their history so far as it is known may be found in a typewritten thesis filed in the library of the University of Illinois. The organisms are numbered throughout the text to correspond with the numbers of the accession list.

- |                              |                                 |
|------------------------------|---------------------------------|
| 2. <i>Sclerotium rolfsii</i> | 8. Pink yeast                   |
| 3. Actinomyces               | 9. White yeast                  |
| 4. <i>Helminthosporium</i>   | 10. White yeast                 |
| 5. Pink <i>Fusarium</i>      | 11. <i>Penicillium glaucum</i>  |
| 6. <i>Rhizopus nigricans</i> | 12. Bacterium                   |
| 7. Actinomyces               | 13. <i>Penicillium italicum</i> |

14. *Penicillium digitatum*
25. *Helminthosporium*
26. *Helminthosporium*
29. *Alternaria*
30. *Mucor*
31. *Bacteria*
32. *Bacteria*
33. *Bacteria*
34. *Gliocladium*
35. *Colletotrichum lindemuthianum*
36. *Helminthosporium*
37. *Helminthosporium*
39. *Sterigmatocystis*
40. *Alternaria*
42. *Penicillium*
44. *Actinomyces*
45. *Bacterium*<sup>1</sup>
46. *Acrothecium*
50. Unknown organism
53. *Lactobacillus*
58. *Azotobacter*
61. *Acrostalagmus cinnabarinus*
62. *Cytosporium ribis*
68. *Fusarium coeruleum*
70. *Sporotrichium bombycinum*
71. *Sclerotinia libertiana*
72. *Botrytis*
73. *Fusarium lini*
74. *Bacillus carotovorus*
77. *Fusarium lini*
86. *Bacillus proteus*
87. *Sarcina lutea*
88. *Sarcina aurantiaca*
89. *Pseudomonas violaceus*
90. *Bacillus prodigiosus*
91. *Bacterium alcaligenes*
92. *Bacillus capsulatus*
93. *Actinomyces albus*
94. *Bacillus ramosus*
97. *Bacillus vulgatus*
98. *Bacterium megatherium*
100. *Cladothrix dichotoma*
103. *Fusarium*
104. *Helminthosporium sativum*
105. *Fusarium lini*
106. *Pilobolus-like fungus*
108. *Alternaria*
110. *Zygorhynchus*
111. *Syncephalastrum*
112. *Cunninghamella*
113. *Rhizopus nigricans*, plus race
114. *Rhizopus nigricans*, minus race
115. *Actinomyces tricolor*
116. *Actinomyces albus* var. *ochraceus*
117. *Actinomyces nigrificans*
118. *Phyllosticta solitaria*
120. *Fusarium culmorum*
121. *Gloeosporium piperatum*
122. *Colletotrichum nigrum*
123. *Ustilago violacea*
124. *Ustilago violacea*
125. *Alternaria crassa*
127. *Bacillus mesentericus*
128. *Helminthosporium*
129. *Fusarium lini*
130. *Fusarium lini*
131. *Fusarium lini*
132. *Pythium*

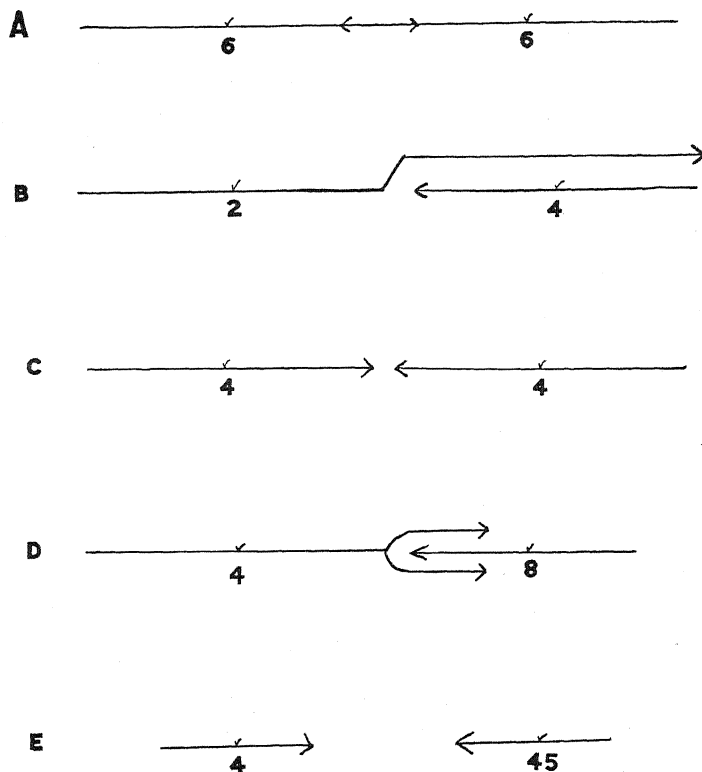
#### TYPES OF INHIBITION

There are many conceivable reactions when the mycelium of one fungus approaches that of another. These theoretical possibilities are nearly all demonstrated by actual examples. Moreover, these examples occur frequently enough and, under properly controlled experiments, regularly enough, to suggest that they may be classified into types which occur whenever the same organisms are made to react with each other. For convenience in discussion, I have described the most prominent and common types of reaction and have designated them by letters.

*Type A* (text fig. 1, A). Mutually intermingling. It might be assumed that this would be a common type, but few really good examples are found. Perhaps the best is afforded by the intermingling hyphae of two colonies of *Rhizopus*. Organism no. 5 grown against no. 5 also presents a fairly good example of this type. It seems, however, but rarely possible for two organisms to occupy equally well the same area at the same time.

<sup>1</sup> The index number of this organism was determined and reads 5131-52120-1333.

*Type B* (text fig. 1, *B*). Growth superficial over the contending organism. The underlying organism is always greatly inhibited. An example is supplied by organism no. 2 grown with no. 4. No. 4 is the inhibited organism.



TEXT FIG. 1. Types of inhibition. *A*, mutually intermingling. *B*, overgrowing. *C*, slight inhibition. *D*, growth around. *E*, inhibition at a distance.

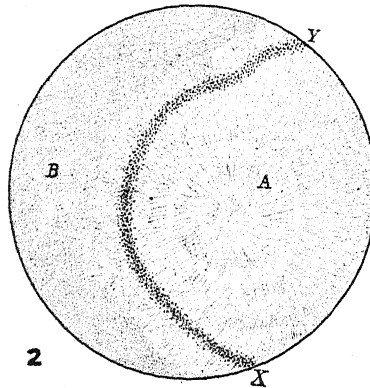
*Type C* (text fig. 1, *C*). Slight inhibition. Both organisms are inhibited but approach each other until almost in contact, when growth ceases. In such cases the space between the two colonies, while very narrow, is clearly marked. This is the prevailing type when any organism is grown with another individual of the same species.

*Type D* (text fig. 1, *D*). Growth around the contending organism. The *Helminthosporium*s react in this manner when grown with organisms numbers 8, 9, or 10.

*Type E* (text fig. 1, *E*). Mutual inhibition at considerable distance. Organisms nos. 31, 33, and 34 so inhibit any of the *Helminthosporium*s. These three organisms when grown with organisms nos. 5, 30, 32, and 35 produce this type of inhibition. The power to inhibit strongly which is

shown by nos. 31, 33, 34, and 45 will be a fruitful field of study for future investigations. They can inhibit growth in many fungi at a distance of 100 mm., and perhaps at greater distances.

Very often a fungus may show for a time complete checking of growth when in the presence of another fungus. Later, growth will appear to be resumed. This growth on closer examination will be seen to be in the deeper part of the medium, which is apparently less affected by the inhibiting organism. The surface growth when once checked usually does not again resume activity. A case of this sort is illustrated by text figure 2.



TEXT FIG. 2. *A*, colony of *Alternaria*. *B*, bacterial growth. *XV* represents the line upon which the *Alternaria* colony was temporarily inhibited.

#### COLONY CHANGES

Inhibition modifies a fungus in many ways evident to the eye. Physiological changes also probably occur, but these will not be discussed at this time. Changes that occur vary directly with the inhibition. All organisms that do not overspread the surface of the contesting fungus are restricted in growth. *Helminthosporium* when alone in a plate would normally continue to grow until the surface of the medium is occupied. If any other organism is placed in the plate with the *Helminthosporium*, the growth of the latter is greatly restricted. Two spores of *Helminthosporium* may germinate in the same plate and the resulting colonies may each cover approximately half the area, with a narrow but definite streak of unoccupied agar between them. If organism no. 31, no. 33, or no. 34 is planted in a petri plate with *Helminthosporium*, the latter forms a colony with a curved margin, the concave side toward the antagonistic organism.

Some fungi are almost entirely inhibited in growth by the presence of other organisms in the same petri plate. This reaction is illustrated by organisms nos. 3, 7, 8, 9, or 10 in the presence of *Helminthosporium*, or of any other fungus that develops mycelium rapidly. Thus we see that antagonism greatly modifies the shape and size of the colony.

Changes in color are quite often associated with other inhibitional changes. Color may be limited to a very narrow band or may pervade the entire mycelium.

Organism no. 12, a bacterial form, causes a narrow (1-mm.) line along the proximal border of an *Helminthosporium* colony with which it is grown. Organism no. 5 causes a similar red line to appear in a colony of organism no. 4 when these two are grown together in a petri plate. Organism no. 5, when grown by itself, has a light pinkish tinge. The depth of this color is modified by the organism grown with it; it may fade out entirely or become bright red, with all gradations between according to the species of the antagonistic organism.

Antagonism stimulates spore-production. Along the line of impending contact between colonies spores are more numerous than elsewhere. No case was observed in which spore-production was decreased, although such spore-production is more apparent with some combinations than with others.

#### MORPHOLOGICAL CHANGES

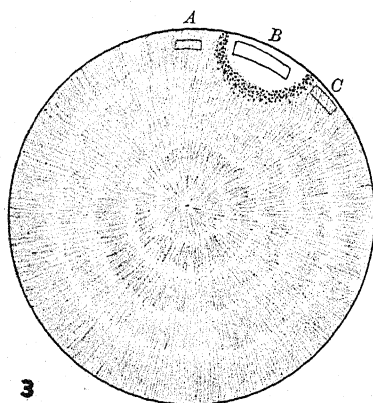
When *Helminthosporium* is growing in pure culture on cornmeal agar under normal conditions of moisture and temperature, the aërial mycelium at the edge of the colony is of uniform diameter, infrequently branched, and straight. If a culture of organism no. 45 is introduced several centimeters away from the periphery of the colony, the following morphological changes occur: when the hyphae of *Helminthosporium* are within 2 centimeters of the bacterial colony, growth slackens and eventually ceases. Branches are given off from the hyphae in every direction and these become gnarled and twisted, and bubble-like enlargements of varying size appear in this portion of the mycelium with almost instantaneous suddenness. The swollen segments may be regarded in some cases as extremely dwarfed branches, but often they appear as enlargements in the existing hyphae. So great is the distortion thus produced that often the species is not recognizable. The multiplication of branches piles up the hyphae along the line of impending contact, which appears to the eye as a black streak. The effect is often heightened by excessive spore-formation along this line. In addition to these distortions, the hyphae have a tendency in their general direction of growth to avoid the zone of influence controlled by organism no. 45 and to grow around it. Elliot (12), working with *Alternaria* and a bacterial form which he designated as "X," found distortions practically identical with those which I have described for *Helminthosporium*, and I have obtained similar results with *Alternaria* when inhibited by my organism no. 45. No. 45 produces strong inhibitory effects under the conditions described in all filamentous fungi experimented with except the *Phycomycetes*, which are little affected.

The greater the inhibition, the greater the distortion. Therefore, the greater the distance through which the reaction takes place, the more marked

are the results. The morphological changes grade from changes in form, size, and structure of the hyphae, and change in direction of growth, to mere cessation of growth with hyphal segments becoming progressively shorter.

The morphological changes described above indicate that there are forces operating capable of producing profound effects on the morphology of the organism involved. In every case in which malformations occur, no morphological differences of diagnostic value could be determined. Three hypotheses suggest themselves as to the causes of these variations: (a) The nutrients may be exhausted. (b) The distortions may be due to change in the osmotic equilibrium of the medium induced by the metabolic activities of the growth process. (c) Certain poisonous products may be created by fungous growth capable of producing malformations and creating a zone through which fungous filaments can not pass. Much work would have to be done in order to determine which of these hypotheses best explains the facts. I am not prepared to give a definite answer to the question, but my experiments have thrown some light that may be of aid in the solution.

It has been explained that organism no. 45 inhibits all *Helminthosporium* at a distance of 2 centimeters or more, so that the growth of the latter is checked entirely. If a block of the medium which occupies the space between the two organisms is removed, aseptic precautions being exercised, and placed in another dish occupied solely by an *Helminthosporium* colony, the filaments of the *Helminthosporium* will not pass over the block but will be checked sharply in front of it, with characteristic distortions. That the effect was not due to mechanical blockade was proven when sterile blocks of normal agar were placed in proximity to *Helminthosporium* colonies and were soon overgrown by the advancing hyphae. Furthermore, blocks of agar taken from the vicinity of organism no. 45 caused inhibition with effects as marked as before when the blocks were inlaid rather than placed upon the surface (text fig. 3).



TEXT FIG. 3. Colony of *Helminthosporium* sharply inhibited in front of B, a block of sterile agar taken from the vicinity of organism no. 45. Blocks A and C are from sterile agar plates and cause no inhibition.

To note the effects of certain chemicals on the growth of *Helminthosporium* filaments, the following substances were used:

1. Copper sulphate crystals,
2. Phenol crystals,
3. Phenol (melted) full strength,
4. Phenol (melted) one-half strength,
5. Chloramine T crystals,
6. Sodium nitrate,
7. Mercuric chlorid,
8. Glucose,
9. 95 percent alcohol (one drop),
- 10, 11, 12, 13, 14. Aqueous solutions of copper sulphate of the following strengths: saturated, 75 percent, 50 percent, 25 percent, and 1 percent.

These materials were placed within 2 centimeters of vigorously growing colonies of *Helminthosporium* no. 20. The chemicals were permitted to act separately in separate plates. Total inhibition was obtained by the use of substances 1, 2, 3, 4, 5, 7, 10, 11, 12, and 13. With substances 6 and 14, slight inhibition was obtained. Substances 8 and 9 caused no inhibition. Where inhibition occurred, except with 6 and 14, distortion of the mycelium was apparent. The distortion was in direct ratio to the strength of the substances used. The distortions were very similar to those previously described when organism no. 45 was used as the inhibiting agent. Those occurring with the use of chemicals very often showed hyphae peculiarly twisted into tight loops. It will be noted that in this experiment those chemicals which are known as powerful germicides and fungicides produced the more marked effects, even though considerably diluted.

#### EFFECT OF MODIFICATIONS UPON INHIBITION

To this point, the phenomena peculiar to antagonism have been considered as occurring under uniform conditions. In order to discover whether variation of conditions materially changes the nature and degree of inhibition, modifications in technique were introduced.

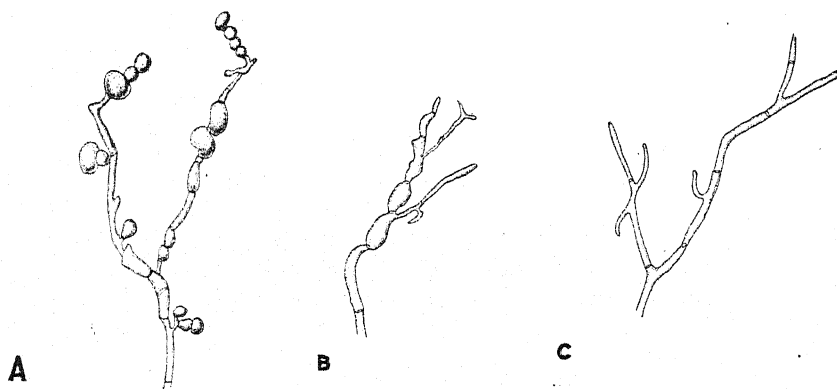
The depth of the medium in a plate was easily varied through all gradations by the simple expedient of pouring the medium in the plates while they were tilted. A colony of a fungus planted in such a plate tended to occupy all sections, and the effect of the presence of other fungi could be tested in both the shallow and the deep portions. Media including cornmeal agar, dextrose agar, plain agar, and washed agar were experimented with in this manner. In all cases in which inhibition occurred normally it was more marked in the shallow portion of the plate than in the deep portions. Evidently the normal inhibitional effect was heightened by the lack of nutrients in the shallow situations. Furthermore, in a normally poured plate the effect of surface inhibition is often overcome by the inhibited fungus sending forward hyphae in the deeper portions of the medium where the inhibitory products make their presence felt to a less degree. In a



shallow medium, however, the inhibitory products penetrate throughout the layer of the medium and the fungus is checked entirely. While the effects are more marked in the shallow portions, they are always the same in character and the inhibitional type is not changed. Spore-formation was more abundant in the shallow portions, and especially in the direction of approaching contact, than was normally the case.

Two types of media were used: one rich in nutrients, the other poor in nutrients. Cornmeal, dextrose, and brazil-nut agars represent the former type, while plain and washed agars are included in the latter class. Many organisms were grown on these media in a manner to test their inhibitional characteristics. The organisms included *Helminthosporium*, bacteria, *Mucors*, *Alternarias*, and yeasts. It was found that, when a fungus was sharply inhibited on normal cornmeal agar, it was better able to contend with its antagonistic neighbor on a medium rich in dextrose. On the other hand, in washed agar the inhibition became even more marked. On media poor in nutrients, the line of demarcation as between two closely related *Helminthosporiums* became much more distinct and had the same characteristics that it would have on cornmeal agar between two widely divergent forms.

The longer a colony of any organism grows by itself on the surface of a nutrient medium, the larger and more vigorous it becomes. Such a large colony is just as effectually checked by the proximity of another organism as if the two contending forms were of equal age and vigor. *Helminthosporium* with a 120-hour start is inhibited by organisms nos. 31, 33, 34, and 45, and the inhibition is of the same type as if they had been planted simultaneously.



TEXT FIG. 4. A, distortion of an *Helminthosporium* filament caused by the presence of organism no. 45. B, distortion of an *Helminthosporium* filament caused by *Bacillus ramosus*. C, a normal filament of *Helminthosporium*.

Change in the mass of inoculum has much the same effect as difference in the time of inoculation. If the mass of inoculum is small, the inhibition

may not be as great and the reaction may be slower. There are exceptions to this statement. The smallest amount of inoculum possible, using organisms nos. 31, 33, 34, and 45, produces the same effect as when a mass many times larger is used.

#### INHIBITION CHARACTERISTIC OF VARIOUS GROUPS

The great groups of fungi were studied critically to ascertain whether any of the types of inhibition previously described were peculiar to them.

Thirty-five different species representative of the Schizomycetes were studied. The Schizomycetes are quite variable regarding the nature of their inhibitions. Some of the most powerful inhibitors belong to this class. These cause marked morphological disturbances and may do so even over considerable distances. On the other hand, most of the bacteria studied were quite inert, causing no inhibition, and being covered eventually by the hyphae of even slow-growing filamentous fungi. It appears from my experiments that the spore-formers as a rule are strong inhibitors. Actinomyces likewise exhibited strong inhibitory action against most filamentous fungi.

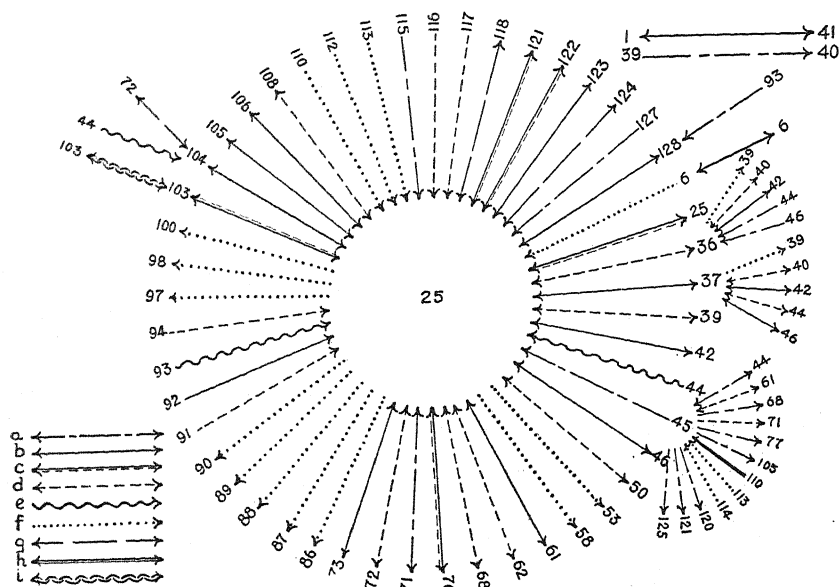
The Phycomycetes have a tendency to grow superficially and to spread rapidly over the surface, burying all other fungous colonies beneath their advancing mycelium. As a rule, they neither cause inhibition, nor are they inhibited.

The Ascomycetes vary somewhat in the nature of their behavior toward each other and in the presence of other organisms. As a rule their inhibitory powers are not great, though some of the yeasts rank high as inhibitors in this class. With respect to these powers no distinction could be made between the conidial and ascigerous forms of the same species.

Few cultures of Basidiomycetes were used because of the difficulty in getting them to grow well in artificial media. Sporidial cultures of two varieties of *Ustilago violacea* were found to be absolutely inert with respect to their inhibitory powers. Moreover, they were but little affected in the presence of other fungous forms, but were invariably overgrown by them.

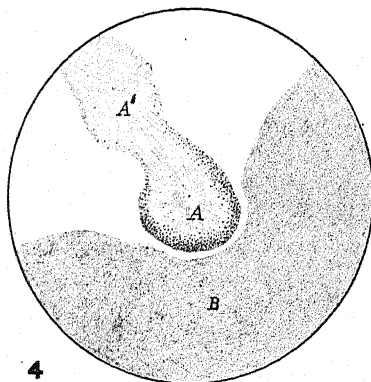
The Fungi Imperfecti, like the Ascomycetes, are quite variable respecting their inhibitory powers. While these powers never appear to be great, yet they are possessed by nearly all of the group to some degree.

Text figure 5 illustrates the nature of the contacts when the various fungi were grown with each other. With but few exceptions, the closer the degree of relationship the less marked is the inhibition between the colonies. If two colonies of *Helminthosporium* grown from spores obtained from a single pure culture are permitted to grow in the same petri dish, they will eventually grow together with their fibers intermingling, giving only slight evidence of separate contact borders. Two colonies of the same species of *Mucor*, if they are of the same race, react similarly, as do also two colonies of *Alternaria*, of *Acrostalagmus*, of *Botrytis*, or of *Fusarium*. If the or-



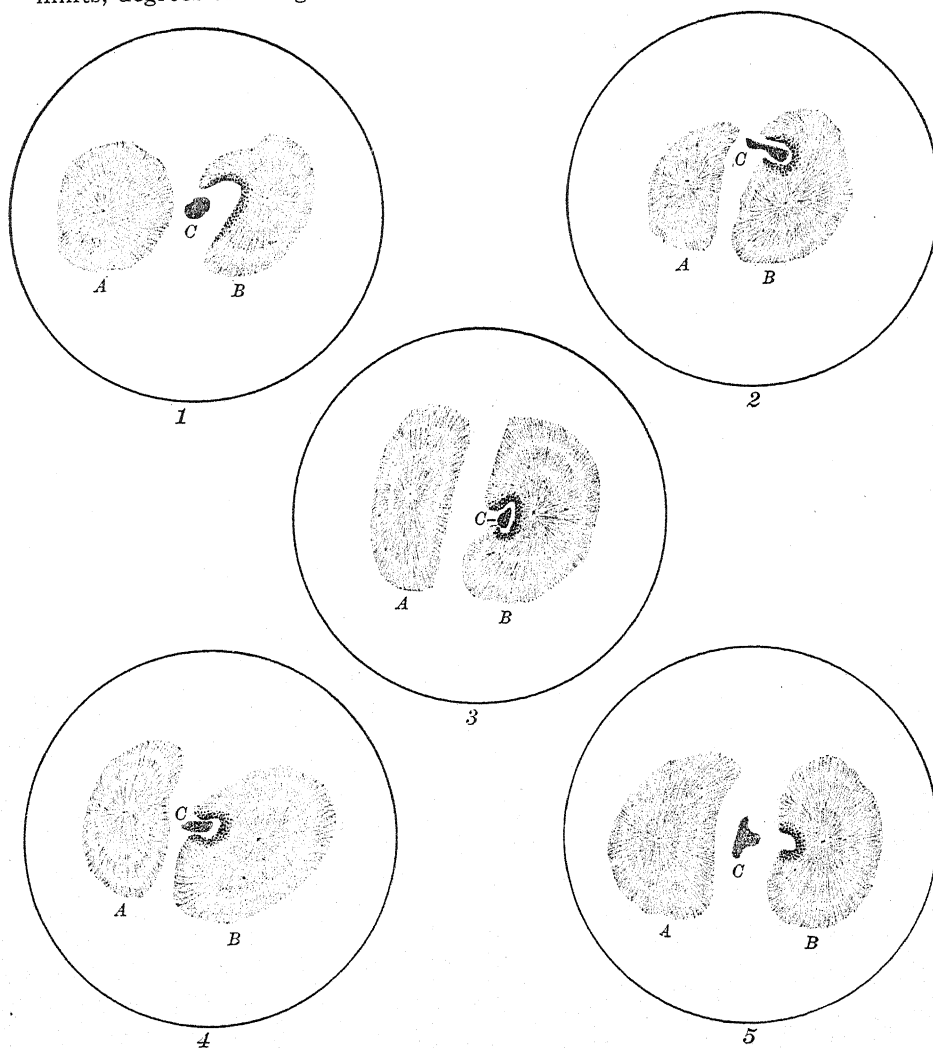
TEXT FIG. 5. Reactions of fungi growing together on cornmeal agar. The figures refer to numbers in the accession list. The arrows point to the direction of the reaction. The symbols are explained as follows: *a*, Antagonistic and producing distortion. *b*, Slightly antagonistic. *c*, Antagonistic at first only. *d*, Complete inhibition without distortion. *e*, Spore-formation increased. Growth checked. No distortion. *f*, Overruns. Arrow points to the colony overrun. *g*, No inhibition. *h*, Grows around. Arrow points to colony grown around. *i*, Mycelium more abundant and raised at contact.

ganisms are of distinct species, they usually betray that fact by some more or less distinct hiatus at contact such as distorted hyphae, color lines, more abundant sporulation, or a wider neutral zone through which the hyphae of the two colonies are unable to pass.



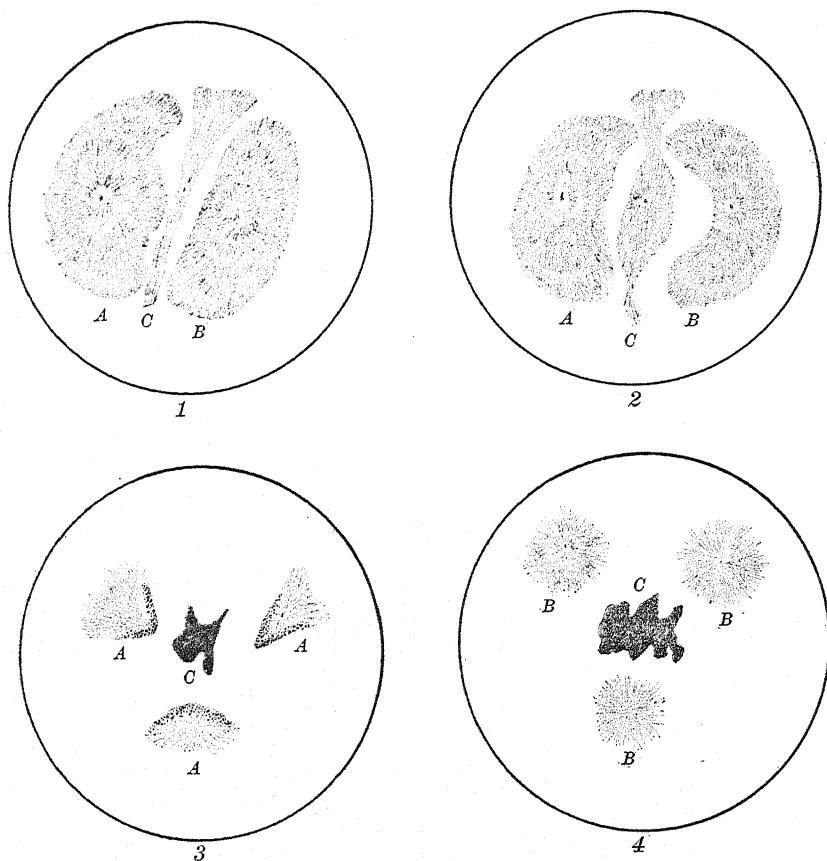
TEXT FIG. 6. *A*, parent colony of *Alternaria*. *A'*, sub-colony of *A*. *B*, bacterial form inhibiting *A*. No inhibition between *A* and *A'*.

When the relationship is more distinct than that of species, the line of demarcation is usually still more marked. Text figure 6 illustrates this point. In this figure, *A* represents a parent colony of *Alternaria*. *A'* is a sub-colony of *A*, and they grow together, merging so perfectly that it is impossible to tell where one colony leaves off and the other begins. On the other hand, the line between *A* and *B*, a bacterial form, is made clearly evident by the increased sporulation at contact. The rule cannot be applied further than this, since one can not say that members of two orders are more antagonistic than are the members of two families. After reaching certain limits, degrees of antagonism are not distinct.



TEXT FIG. 7. In every case *A* represents organism no. 25; *B* represents organism no. 26. In 1, *C* = organism 31; in 2, *C* = 8; in 3, *C* = 32; in 4, *C* = 12; and in 5, *C* = 10.

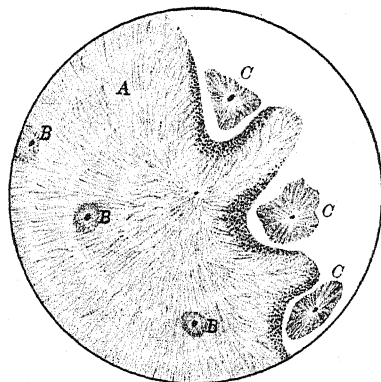
Two different fungi may not respond alike in the presence of other forms. This may be of diagnostic aid even when the fungi concerned are as closely related as varieties of the same species. Two varieties of *Helminthosporium teres*, nos. 25 and 26 of the accession list, illustrate this possibility. The colonies of these *Helminthosporium*s differ somewhat in appearance, zonation being more marked in the latter than in the former. The organisms were both inhibited by a number of organisms with which they were grown, but reacted differently with most of them. The difference was so great that one had no difficulty in distinguishing between the two varieties, judging solely from the reactions due to antagonism. Text figures 7 and 8 illustrate the differences described. When organisms nos. 25 and 26 were grown in the presence of organism no. 31, a bacterial colony, no. 25 is not inhibited while inhibition in no. 26 is characterized by cessation of growth and greater spore-formation. In the presence of organism no. 8, a yeast, no. 25 is inhibited somewhat, as may be seen by the fact that toward the yeast colony



TEXT FIG. 8. In every case *A* represents organism no. 25; *B* represents organism no. 26. In 1, *C* = 5; in 2, *C* = 29; in 3 and 4, *C* = 33.

no. 25 presents a straight rather than a rounded outline. No. 26 is inhibited more sharply and with spore-formation increased. In the presence of organism no. 32, a bacterial form, much the same relations exist as with the case of the yeast just described. These relations are also much the same when the two *Helminthosporium*s are grown in the presence of organism no. 12, another bacterial form, or of organism no. 10, a yeast. Both organisms nos. 25 and 26 are inhibited by organism no. 5, a *Fusarium*, although in this case the growth of no. 25 is more sharply checked than is the growth of no. 26. Little difference in inhibition is to be noted when the two are grown together in the presence of organism no. 29, an *Alternaria*. In the presence of organism no. 33, an arborescent-growing bacterium, no. 25 is sharply inhibited at a considerable distance with cessation of growth, distortion of hyphae, and increased spore-formation toward the sides presented to the bacterial colony. On the other hand, no. 26 showed no evidence of inhibition in the presence of organism no. 33. Summarizing, no. 25 is inhibited markedly only by organisms nos. 5, 29, and 33. No. 26 is markedly inhibited by organisms nos. 31, 8, 32, 12, 10, 5, and 29. This experiment was repeated many times, invariably with the same results as described above and as shown by text figures 7 and 8.

Reference to the diagram (text fig. 5) reveals illustrations of the same sort. In this diagram attention is called particularly to reactions exhibited



TEXT FIG. 9. *Pestalozzia* inhibited by C, *Penicillium* colonies, but not by B, *Penicillium* colonies of a species different from C.

by organisms nos. 36 and 37. In this instance *Acrothecium* caused distortion, the threads being twisted into knots when this fungus was in contact with organism no. 36, an *Helminthosporium*; no. 37, another *Helminthosporium*, was slightly inhibited by the *Acrothecium* but without visible distortion. In this case no. 37 caused more abundant spore-formation in the *Acrothecium* colony. *Actinomyces* caused both *Helminthosporium*s to be inhibited, but distortion of filaments was produced only in no. 36. With

organism no. 25, another *Helminthosporium*, growth was completely stopped in no. 36 with practically no intermingling of hyphae. Growth of no. 37 was slowed somewhat in the presence of organism no. 25, but the hyphae mingled to a considerable degree.

Text figure 9 shows a culture of *Pestalozzia*. This became contaminated with two races or species of *Penicillium*. Three colonies of one kind of *Penicillium* caused no inhibition of the *Pestalozzia*. The other *Penicillium*, of which there were also three colonies, completely inhibited the *Pestalozzia* with increased sporulation and a piling up of the hyphae. These two kinds of *Penicillium* are clearly differentiated by the nature of their reaction with the *Pestalozzia*.

#### BIOLOGICAL EQUILIBRIUM

Inasmuch as organism no. 45 was found to possess such extreme inhibitory powers, an effort was made to discover whether this organism might be of some practical importance in checking the growth of other fungi upon plants parasitized by them.

In the first of this series of experiments, sterile wheat seedlings were grown in rag-dolls and the cloth around the seedlings was heavily inoculated with *Helminthosporium* spores. One half of the rag-dolls prepared in this manner had previously been immersed in a broth culture of organism no. 45. Table I gives the results of this experiment.

TABLE I. *Protection afforded wheat seedlings from attacks by Helminthosporium, using organism no. 45 as the protecting agent*

	October 25			November 3			December 2			December 18		
	Infected	Uninfected	Percent Infected	Infected	Uninfected	Percent Infected	Infected	Uninfected	Percent Infected	Infected	Uninfected	Percent Infected
Protected by no. 45....	1	9	10	1	9	10	14	28	33.3	3	20	13.04
Unprotected.....	4	6	40	5	50	28	15	65.1	20	9	9	68.9
Without either <i>Helminthosporium</i> or organism no. 45.....	0	10	40	0	10	0	0	35	0.0	0	25	0.00
Without <i>Helminthosporium</i> , with organism no. 45.....	0	10	0	0	10	0	0	36	0.0	0	30	0.00

The percentages given in this table would indicate that a certain amount of protection was given to the wheat seedlings by the presence of organism no. 45.

The next experiment was made to determine whether organism no. 45 would protect plants under more natural conditions than existed in the foregoing experiment. It was decided to attempt the protection of flax seedlings grown in pots from attacks of *Fusarium lini*. *Fusarium lini* grows



well in the soil, quickly infects and kills the plants, and in plate cultures was inhibited by organism no. 45, although not so strongly as are the *Helminthosporiums*. In this experiment the protected seedlings had their bases surrounded by cores of earth heavily impregnated with a broth culture of organism no. 45 and separated from the surrounding earth containing *Fusarium lini* by paper cones. The controls were surrounded by cores of sterile earth, also in paper cones. The earth in these cones was saturated with a sterile broth solution in order to have the physical conditions the same. All the plants in this experiment succumbed to *Fusarium* wilt. The controls, however, in every case showed indications of the wilt before the protected plants did. An examination of the protected plants indicated that the roots had penetrated the paper cones and were growing into the unprotected regions containing *Fusarium*. This fact probably accounts for the absence of protection exhibited by the inhibiting organism in this case. Further experimentation on this phase has been inconclusive because of difficulty in securing virulent strains of *Fusarium lini*.

Experiments were made to determine whether organism no. 45 was as strongly inhibitory to *Fusarium lini* in soil as on culture plates. Soil thoroughly moistened with beef bouillon was packed into test tubes. The lower third of the tube was inoculated with *Fusarium lini*, the middle third was moistened with a broth culture of organism no. 45, and the top third was sterile earth. In the controls, the middle third of the tube was filled with sterile earth. Samples were removed from time to time from the top third of each tube and plated. After a month, *Fusarium lini* had not penetrated to the top third of earth in any tube where the intervening third was impregnated with a suspension of organism no. 45. In most of the controls, after an interval of two to three weeks *Fusarium lini* could be detected by cultural methods in the upper third of earth.

Plate cultures of earth were made, the earth being moistened with beef bouillon and then packed while damp into plates to the depth of a half centimeter or more. Transfers were made from pure cultures of fungi to the surface of the earth on these plates in the same manner as one would inoculate plates of agar. Most fungi grew nearly as well, although perhaps more scantily, on such plates as on plates containing cornmeal agar. *Fusarium lini* was as sharply inhibited by organism no. 45 on such a plate as on ordinary media. In fact, all fungous combinations tried reacted in much the same way as illustrated in text figure 5. On plain earth moistened with sterile water, most fungi grew so scantily that their inhibitions could only with difficulty be determined. On non-enriched earth, organism no. 45 grows so slowly that it is doubtful whether it would be very useful in checking the normal soil flora.

Potter's (29) suggestion that plants may be protected from fungous attacks by injections of the metabolic by-products of the fungus concerned was thought worthy of testing at this time because it supports the idea that

inhibition is due to products secreted or excreted by the fungus rather than to the exhaustion of nutrients. Oranges were injected with the by-products of the growth of *Penicillium* and were then inoculated with *Penicillium*. Growth of the *Penicillium* was sharply inhibited at the line of injection and but slowly and imperfectly worked its way over the line. Oranges that had not been injected with the staling solution were quickly and entirely covered with the *Penicillium*.

Since there exists within the soil such a very close association between rootlets and fungi, the possibility exists that one may be very much affected or even considerably modified by the other. In order to determine how far this might be true, aseptic seedlings of barley, oats, wheat, and rye were placed in close proximity to colonies of *Helminthosporium* and of bacteria growing in petri dishes upon cornmeal agar. The seedlings were placed in such a manner that the tips of the rootlets of one and the sides of the rootlets of the other were presented to the fungous colony. The following possibilities were kept in mind: (1) Inhibition or stimulation of the fungus; (2) Inhibition or stimulation of root-hair production; (3) change of direction of growth of the rootlet tip. After exposure for 48 hours there were no noticeable effects either upon the fungus or upon the rootlets (Plate VI, fig. 2).

#### DISCUSSION

Smith (40) summarizes the possibilities existent when two or more organisms are grown in close proximity as antagonistic, indifferent, or favorable. Zeller and Schmitz (48) enumerate the possibilities as stimulating, inhibiting, overgrowing, and non-influencing.

Garré (15), de Freudenreich (10), Laws and Andrews (21), Remy (37), Horrocks (19), and Frost (13), all working with *Bacillus typhosus*, have demonstrated that it may be inhibited in the presence of several other bacterial organisms.

The antagonism of protozoa toward bacterial forms has been put to practical use in the purification of water and sewage. In this instance, however, the inhibitory action may be due to the actual ingestion of the bacteria by the other organisms involved. Purdy and Butterfield (32), Razetto (35), Huntmuller (20), Stokvis (45, 46), and Olitsky (27) have cited such examples.

It has frequently been demonstrated during my experiments that some of the most profound inhibitory effects are exhibited by bacteria toward fungi. This fact has been noted by Ravn (33, 34), Elliot (12), and Reinhardt (36).

The antagonism which certain fungi exhibit against other fungi of the same or of different species has been noted in the literature by Blakeslee (3), Edgerton (11), Stevens and Hall (42, 43), Crabill (8), Reinhardt (36), Fulton (14), Zeller and Schmitz (48), F. L. Stevens (41), N. E. Stevens (44),

and among the higher fungi the same phenomenon is noted by Shantz and Piemeisel (38).

The references to well authenticated instances in which fungi have stimulated growth of some sort or have been otherwise beneficial to each other are not so numerous. Nevertheless, such references are made by F. L. Stevens (41), Stevens and Hall (43), Shear (39), Zeller and Schmitz (48), Reinhardt (36), Ward (47), de Bary and Woronin (9), Manns (24), Pringsheim (31), and Nikitinsky (25).

These effects, both antagonistic and stimulative, have a very direct bearing upon the members of a mixed culture and the nature of succession in such a culture. Pringsheim and Nikitinsky have expressed such views in articles already cited (31, 25). This idea is also upheld by Gwynne-Vaughan (16), Hesler (18), Smith (40), and Heinemann (17).

The explanation of effects produced upon fungi in mixed cultures may be divided into two classes: (1) The nutrients of the medium may have become exhausted; (2) Products are formed which are detrimental or beneficial to further growth. Obviously when the nutrients are exhausted growth will be checked, but Leisegang (22) is one of the few who would explain the entire inhibitional phenomenon in this manner. On the other hand, many advocate the theory that during growth organisms give off materials that may be inhibitory or stimulatory to themselves or to other members of the flora. Gwynne-Vaughan (16), Clark (7), Brown (4), Balls (1), Fulton (14), Lutz (23), and Chambers (6) are advocates of the latter theory.

By taking advantage of the products produced as mentioned in the preceding paragraph, fungous growth may be inhibited or stimulated to the advantage of man. Those who have done this experimentally are Potter (29, 30), Picardo (28), and Beauverie (2). Norton (26) believes that such experiments have no practical value.

Judging from my own experiments and from the literature on this subject, it would seem possible that under certain circumstances the knowledge of the relationships of fungi could be used in controlling the growth of these organisms to our advantage.

#### SUMMARY

1. The inhibitions exhibited by fungi may be grouped into five classes.
2. *Helminthosporium* was inhibited by various chemicals in a manner similar to that caused by other fungi.
3. The inhibiting qualities of a fungus may be of aid in identification of species.
4. The richer the medium in nutrients the less marked were the inhibitions.
5. The inhibitions varied but slightly with changes in the amount of inoculum, in time of inoculation, or in depth of medium.
6. A common cause of the inhibitory action in the cases studied was determined to be the presence of some product formed during growth.

7. Seedlings were protected measurably from infection by *Helminthosporium*, using organism no. 45.

8. Flax seedlings were measurably protected from *Fusarium*, which could only with difficulty pass a layer of earth heavily infected with the inhibitor.

9. Roots of seedlings and root hairs gave no tropic response in the presence of fungi.

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## EXPLANATION OF PLATES

## PLATE IV

FIG. 1. *Alternaria* inhibited by two colonies of organism no. 45.

FIG. 2. *Helminthosporium* in the center inhibited on three sides by *Actinomyces*.

FIG. 3. *Helminthosporium* in center inhibited by four small colonies of organism no.

45.

## PLATE V

FIG. 1. Nature of reaction when two colonies of the same species grow together. Organism no. 25 of the accession list.

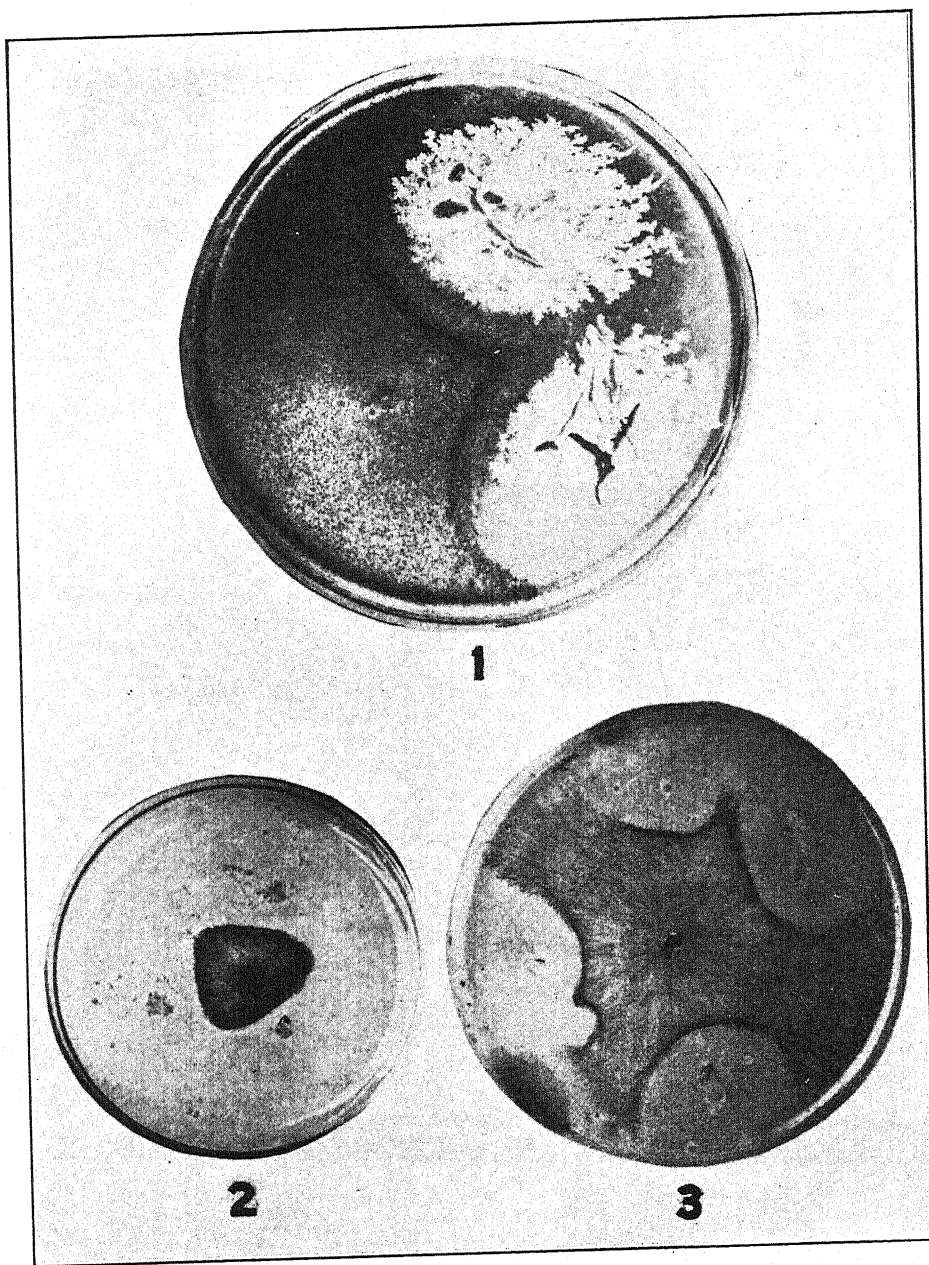
FIG. 2. Nature of the reaction when two species of *Helminthosporium* grow together. Organisms nos. 25 and 37 of the accession list.

## PLATE VI

FIG. 1. *Helminthosporium* inhibited by a block of sterile agar taken from the vicinity of organism no. 45.

FIG. 2. Indifference of wheat rootlets to a colony of *Helminthosporium*.

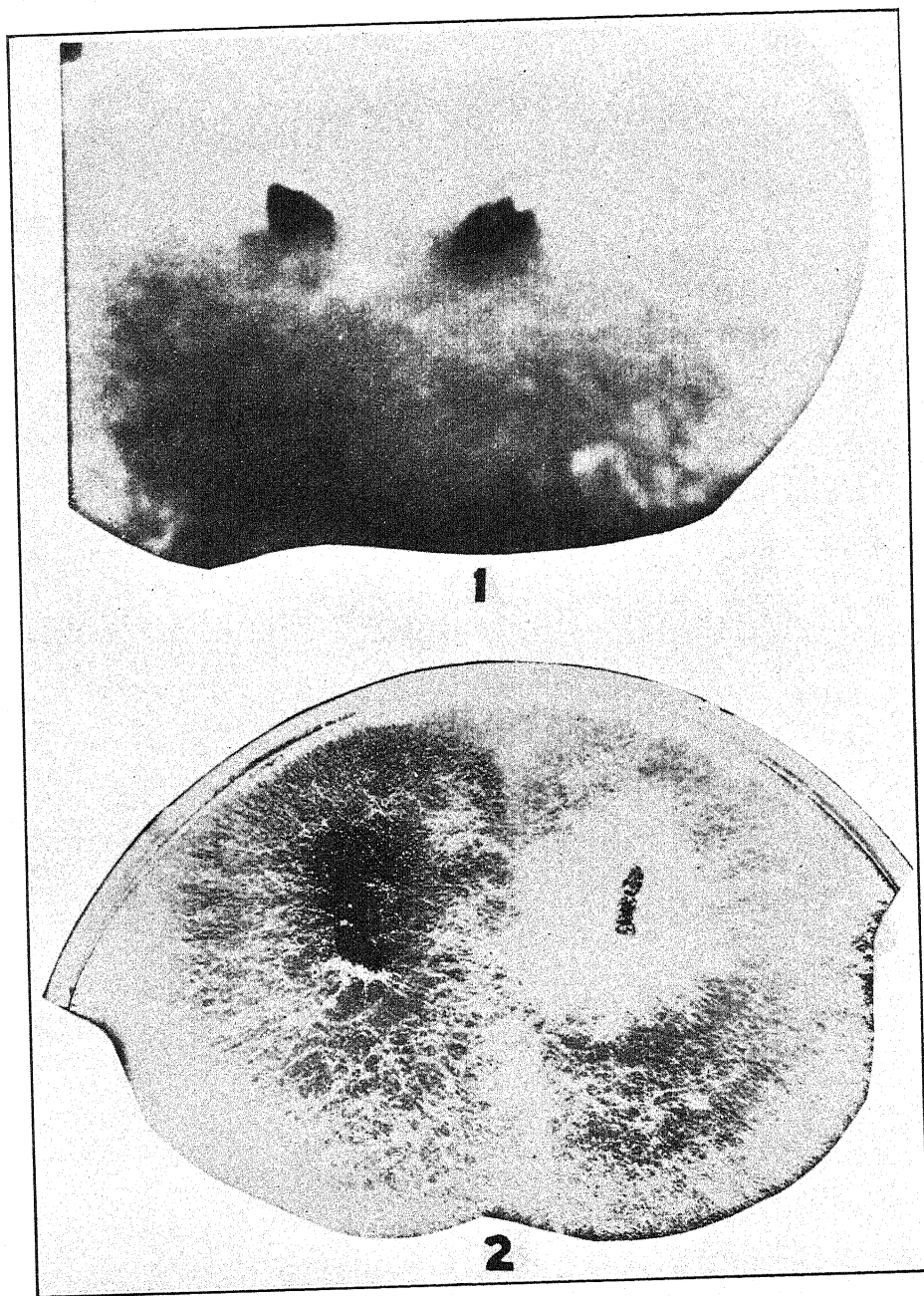




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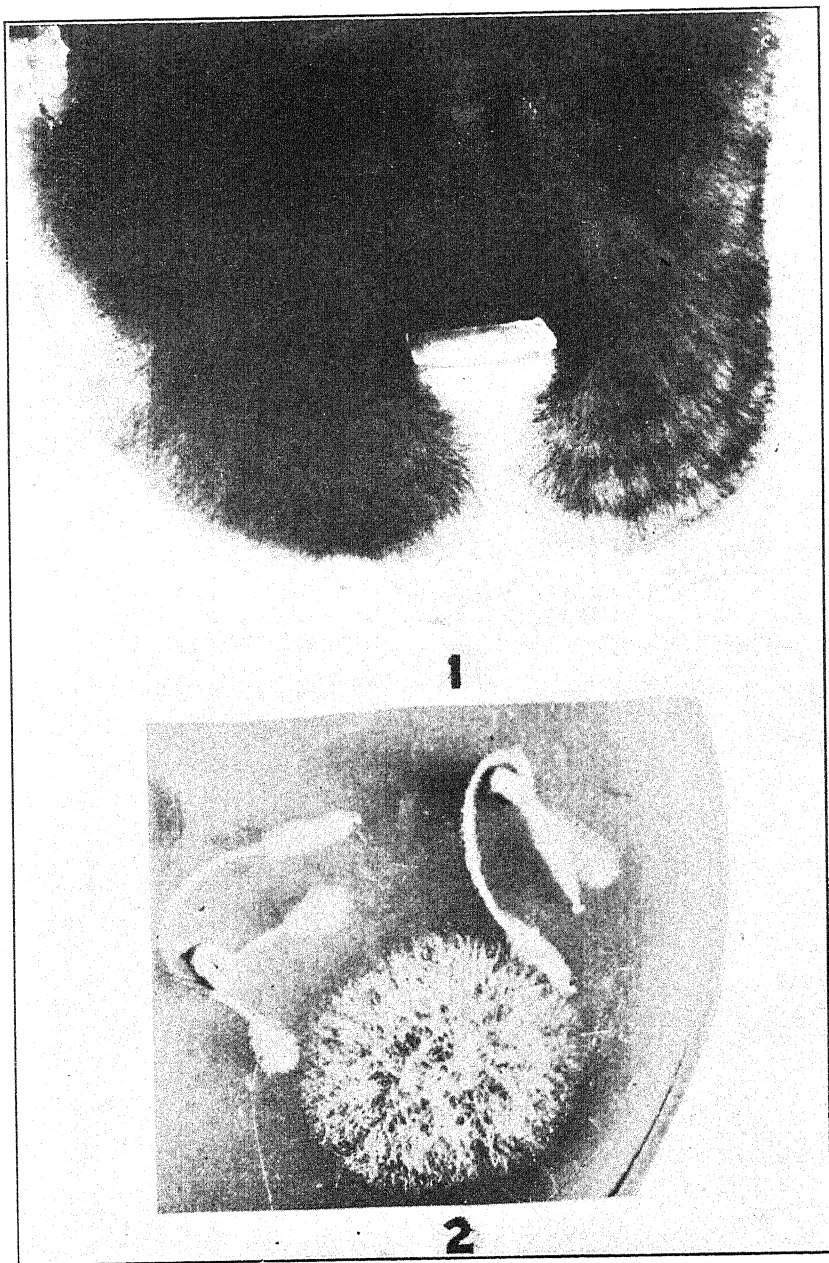






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## FACTORS INFLUENCING INFECTION OF *HORDEUM SATIVUM* BY *USTILAGO HORDEI*<sup>1</sup>

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### INTRODUCTION

The classical researches of Tulasne (53, 54), Kühn (31), Fischer von Waldheim (15-17), Brefeld (3-6), and Jensen (25, 26) during the latter half of the past century established the essential facts regarding the morphological characters of the fungus causing the covered smut of barley and the etiology of this disease. The thoroughness of the investigations of these earlier workers made possible the development of control measures which, when applied, completely eliminated or reduced to a negligible quantity the devastation by the disease. These control treatments, carefully carried out in the research laboratory, were so effective that they placed the knowledge of the cereal smuts in general upon a more satisfactory basis than had been reached in any other comparable group of plant diseases. The development of the hot-water and formalin seed treatments for the control of the seed-borne smuts was followed by extensive experiments to perfect the use of these preventive measures. At the same time exhaustive researches were made to secure methods of seed treatment which would be less difficult in their application.

Reports of the National Plant Disease Survey (32-37, 48) have revealed the fact that enormous losses are being sustained yearly from the smut diseases. That seed treatment is far less practiced than has been generally supposed is made apparent by the results of these surveys. This knowledge, coupled with the results in varietal testing secured by Farrer (13), Tubeuf (52), Rose (45), Sutton (50), McAlpine (39), Kirchner (30), Vavilov (55), and Reed (43, 44), which have demonstrated that some varieties are relatively resistant to smuts, have led many pathologists to believe that the various disinfectants are but temporary measures at best and that permanent relief from these, as from many other diseases, will come through the development of suitable resistant varieties.

The primary consideration in the development of disease-resistant varieties is an understanding of the conditions influencing the unequal infections in varietal tests, and with this in mind the present investigation upon the covered smut of barley (*Ustilago hordei* (Pers.) Kell. and Sw.) was undertaken.

<sup>1</sup> Contribution from the Department of Botany of Columbia University no. 324. Brooklyn Botanic Garden Contributions no. 35. Published, at the expense of the Brooklyn Botanic Garden, out of the order determined by the date of receipt of the manuscript.



## THE FUNGUS

## Historical

Cultivated barley is commonly attacked by two smuts; (1) the loose smut (*Ustilago nuda*) in which the spores are distributed during the flowering of the host, and (2) the covered smut (*Ustilago hordei*) in which spore-distribution takes place mainly at the time of threshing the grain.

It is quite evident from their spore-germination and seed-inoculation results that some of the earlier writers were dealing with *Ustilago hordei* (Pers.) Kell. & Sw. However, the results are confusing and the statements of uncertain application until the work of Jensen (25), who pointed out differences between the appearance of the smut diseases of barley which made it possible to state what disease was under observation. Jensen, owing to his inability to secure infection when he inoculated oats, wheat, or barley with spores from either of the other two, stated that these smuts are biologically different varieties, perhaps even distinct species. At this time he separated smut on barley into two forms, for which he proposed the name *Ustilago carbo* var. *hordei* f. *tecta* for the organism causing covered smut, and *U. carbo* var. *hordei* f. *nuda* for that causing loose smut. In 1889 he (26) raised these forms to varietal rank, proposing the name *U. hordei* var. *tecta* for the former and *U. hordei* var. *nuda* for the latter. Jensen, according to Kellerman and Swingle (29) also made the interesting observation that solutions which kill spores adhering to the outside of the grain do not prevent loose smut. From this he concluded that the infective material of the loose smut is internal and not external to the seed grain.

Brefeld (4), by a series of careful inoculation experiments, came to the conclusion that the loose smuts of wheat and barley are not identical with the smut on oats, and therefore accorded these loose smuts specific rank. He proposed the name *Ustilago hordei* for the fungus causing the smut on barley and the loose smut of wheat, diseases which he supposed to be caused by the same organism. Although Brefeld pointed out that the germination of loose smut spores on barley received from Japan was without conidia, a condition quite different from that with which he was at that time familiar in spore-germinations of the *Ustilago carbo* of Germany, he in no way indicated that there might be two different smuts on barley. Jensen's conclusion that the infective material of loose smut is internal and not external to the seed grain was confirmed by the demonstration of flower-infection in loose smut of wheat by Maddox (38) in 1897, Nakagawa (according to Hori, 23) in 1898, and for the loose smuts of both wheat and barley by Brefeld and Falck (9) in 1905, and was further substantiated by the histological studies of Hecke (21) who traced the smut mycelium in the growing points of barley seedlings infected with *Ustilago nuda*.

Rostrup (46) demonstrated that one of the barley smuts (f. *tecta* Jensen) produced smutted grain when spores were dusted on the seed, while the



other (f. *nuda* Jensen) did not. Rostrup called the former *Ustilago jensenii* n. sp., and the latter *Ustilago hordei* Brefeld.

Kellerman and Swingle (29) separated the two barley smuts into species upon the basis of difference in spore-germination and in morphological characters. They noted variations in the manner of spore-germination of the covered smut received from Canada, New York, and Denmark when compared with that of material collected in Kansas, but concluded that these differences were of minor importance. For the fungus causing the loose smut of barley they proposed the name *Ustilago nuda* (Jens.) Kell. & Sw., and for the one causing the covered smut *Ustilago hordei* (Pers.) Kell. & Sw., names which have been generally accepted by most American and many European workers. Some mycologists have retained the names of Rostrup, however, and this has caused uncertainty as to which smut was under observation by certain writers. It is not clear whether Hori (23) in Japan had in mind the covered-smut fungus when he listed *Ustilago hordei* among the cereal smuts which infect the host at flowering time. If he intends to convey this impression, his findings are quite different from those of other investigators, who have found seedling-infection to be the rule in this disease.

The synonymy of the fungus is adequately summarized by Kellerman and Swingle (29), Clinton (12), and others, and will not be reviewed here. However, it seems well to point out that *Ustilago hordei* (Pers.) Kell. & Sw. is *Ustilago jensenii* Rostrup, and that *Ustilago hordei* Brefeld, as interpreted by Rostrup, is *Ustilago nuda* (Jens.) Kell. & Sw. Brefeld applied this name to both smuts of barley and to the loose smut of wheat, and the application of the name to the loose smut of barley by Rostrup was quite a different interpretation from that actually made by Brefeld. Therefore, this name applied to loose smut should be credited to Rostrup and not to Brefeld. The names of Kellerman and Swingle (29) have priority and for this reason should be universally adopted.

A third smut disease of barley was described by Biedenkopf (2) from material collected near Halle, Germany. The fungus causing this malady is described as combining the morphological and germination characteristics of the above-named forms, *i.e.*, echinulate spores germinating by a four-celled promycelium which cuts off conidia. He named this new form *Ustilago medians*. No further reports of this disease are to be found in the literature, and its distribution and importance are unknown.

#### THE HOST

*Ustilago hordei* has a world-wide distribution upon cultivated barley.

For purposes of clearness and brevity in expression, all cultivated barley varieties are here referred to as *Hordeum sativum*, following Jessen (27) in this respect. This species has been divided into four sub-species (19, 58), which have in turn been divided into many forms, varieties, etc. In the

present study the barley is referred to by its varietal name and the seed number, since the recent investigations (30, 55, 43, 44) upon varietal resistance to various cereal smuts have shown the importance of stating the variety and even the selection within the variety if possible.

### Growth and Maturity

Barley is grown as both a summer and a winter annual. In general, the spring varieties are erect as compared with the decumbent habit characteristic of the winter barleys. The winter varieties sown in the spring will not, as a rule, head satisfactorily; the maturity of these varieties can be hastened, however, by germinating the seed at low temperatures. An experiment was conducted in which seed was planted in constant-temperature tanks in a soil uniform as regards moisture, acidity, etc. After the seedlings were two inches high they were transplanted to the greenhouse bench under conditions favorable for barley growth. The greenhouse temperature was maintained between 65° and 80° F.

TABLE 1. *Effect of the Temperature During Germination upon the Growth and Maturity of Texas Winter Barley*

Date of Planting	Temperature during Germination	No. of Days until Plants Headed	Remarks
Feb. 8	7°-8° C.	100	Plants headed and matured uniformly.
" 7	18° C.	126	Plants headed irregularly.
" 7	23° C.	131	Only a few culms headed; the plants continued to tiller profusely and never matured satisfactorily.

The plants germinated at the low temperature produced an average of 1.8 culms which headed evenly and produced normal heads of good, plump kernels. These plants compared satisfactorily in every way with those from seed of this variety planted in the field in the fall, except that fewer tillers were produced.

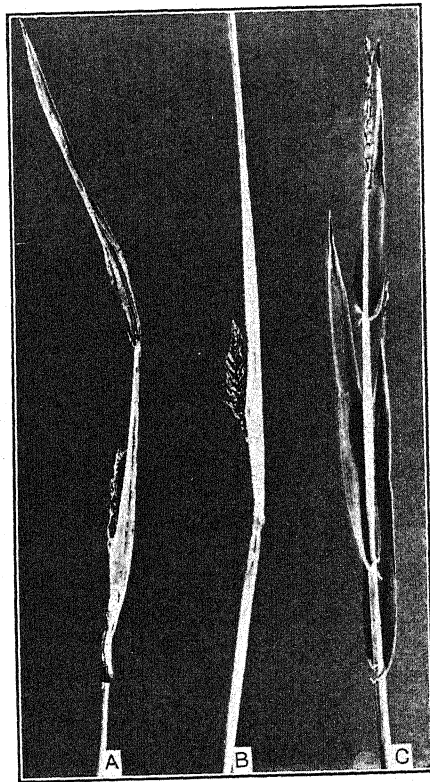
This experiment confirms the observation of Appel and Gassner (1) that low germination temperatures (5°-7° C.) favored early maturity in winter barley, while the same variety failed to mature when germinated at a temperature ranging from 20°-25° C.

In making genetical studies in which crosses between spring and winter varieties are involved, germination at low temperatures in the winter or early spring would seem to be a satisfactory method of avoiding the danger of winter killing on the one hand and, on the other, the uncertainty of the plants maturing if seeded in the usual way. If this relation between germination at low temperatures and maturity holds in the case of winter wheat, it suggests a method of getting around some of the difficulties recorded by Gaines (18) in his studies on the genetics of bunt resistance, and by Hayes and Stakman (20) on rust resistance.

## THE DISEASE

## Symptoms

Plants infected with *Ustilago hordei* are not distinguishable from healthy plants until near the heading time. In severely infected plants the leaf tips of the upper one, two, or three leaves may show smut pustules before the presence of the disease in the head is apparent (text fig. 1, C). Often in such cases long streaks of smut appear on the leaves, even extending to the leaf sheaths and the nodes below (text fig. 1, A). Such cases, while rather rare in the field, have often been found in plants grown in the greenhouse.



TEXT FIG. 1. Covered smut of barley. A. Smut on leaf, leaf sheath, and node. B. Smutted head breaking through the base of the leaf sheath. C. Smut pustules developed at the tips of the last three leaves as well as in the spikelets.

As a rule, a portion of the healthy plants head before any of the diseased ones appear, thus giving the impression at first that all the plants are healthy. In some cases the smutted heads do not push out at the top of the "boot," as do normal heads, but enlarge and eventually burst through the leaf

sheath at the base (text fig. 1, *A*, *B*). This peculiarity is striking in some varieties and under certain conditions, while in other varieties and under other conditions the diseased plants elongate as do the uninfected.

Infected culms remain green one or two weeks longer than do healthy ones and stand up straighter than normal plants in consequence of the light weight of the smutted head as compared with the mature head of well-filled kernels (Pls. VII, VIII).



TEXT FIG. 2. Covered smut of barley. *A*, *B*. Young plants developing smut pustules on leaves, leaf sheaths, nodes, and internodes before heading. *C*. Partially smutted head.

At times partially smutted heads appear (text fig. 2, *C*). In my experiments it has always been the upper parts of the heads in such cases which have remained healthy, the lower parts being diseased. The writer has never seen the reverse, *i.e.*, the lower part healthy and the upper part diseased, in the case of this barley smut. In these partially smutted heads there is invariably a sterile zone between the smutted spikelets below and the plump kernels above (text fig. 2, *C*). The ovaries of the flowers in this zone abort, neither producing barley grains, as do those above, nor large

smut sori, as do those below. Smutted spikelets are usually completely smutted except small portions of the ends of the glumes. The spikelets attached together on the rhachis do not, as a rule, separate, but push out in one large smut mass, which replaces the kernel and adjacent parts. All grades of transition from partially smutted ovaries to entirely smutted spikelets (text fig. 1, *C*) have been found. The smut pustules may continue throughout a part, or even the entire length, of the awn. The awns on badly diseased heads are usually silvery white in appearance, much softer than normal awns, and considerably reduced in length.

#### INFECTION

Owing to the conflicting statements of various authors (4, 18, 31, 51), the place of entrance of the fungus into the seedling cannot be regarded as determined at present. For purposes of these experiments it is sufficient to point out that the work of Appel and Gassner (1) and Schellenberg (47) has demonstrated that seedling-infection does not take place after the first green leaf has pushed one centimeter through the coleoptile. In the temperature experiments herein reported, all plants were held at the stated temperature until they were well beyond this susceptible period.

#### VARIETAL IMMUNITY AND SUSCEPTIBILITY

Covered smut of barley is a serious disease of this cereal crop during some seasons and under certain conditions which are not well understood. However, under field conditions, the reports of the Plant Disease Survey show that this disease causes, at times, very considerable losses. While general state averages (48) for losses due to covered smut are, as a rule, low, individual fields in Texas suffered losses of as much as 60 percent in 1919, in some Missouri fields 30 percent of the plants were smutted in 1921, and in the same year fields in the state of Kansas were reported in which half of the heads were smutted.

Rose (45) reports data bearing upon the susceptibility of 41 varieties of barley to smut. It is not clear whether he was dealing with the loose or the covered smut, but in any case his percentages of infection are low, or negative. Broili (10), Tisdale (48), and Mackie (48) report inconclusive results in varietal tests of barley for the covered smut. In discussing this disease in California, Mackie (48) states that:

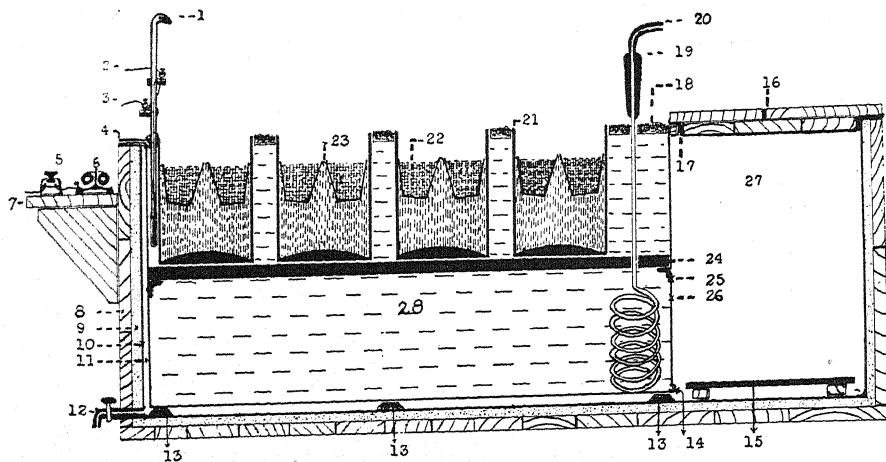
Covered smut is apparently affected by climatic and soil conditions to a marked extent. The low-lying river or lake bottom lands usually show the most barley smut. The percentage of attack appears to be greater in wet years.

#### SOIL FACTORS INFLUENCING INFECTION

In order to determine the conditions necessary for testing the susceptibility of the numerous varieties of barley to the covered-smut fungus, it is necessary to know what environmental conditions favor high infection by

the parasite. Especially is it desirable to know the influence of soil temperature, moisture, and acidity at the time of germination upon infection.

The relation of soil factors, especially soil temperature, to infection by organisms below ground has long been emphasized by Jones (28) and his co-workers. In dealing with these problems they have evolved various forms of a temperature tank for the control of soil temperature. The tanks used in my experiments are based, in principle, upon the Wisconsin apparatus, but considerable modification has been made to meet special conditions. In growing spring grains, which are usually sown in a cool soil, it was necessary to maintain the temperature lower than was possible with running tap water for cooling. This was done by the use of ice, for which the tank had to be considerably modified. For accurate temperature-distribution throughout the tank, a stirring apparatus consisting of a paddle wheel run by a motor, as shown in text figure 4, was installed. The tanks were constructed in units such that each was complete in itself. Each tank was 4 feet in length, 2½ feet in width, and 1½ feet in depth. The outer box of 1½-inch wood was lined with a 1½-inch layer of hair felt, and inside this was placed a 24-gauge galvanized-iron lining. An inner chamber was made to fit this outer one in such a manner as to provide for an ice box at one end and an air space below and at the sides, as shown in text figure 3.

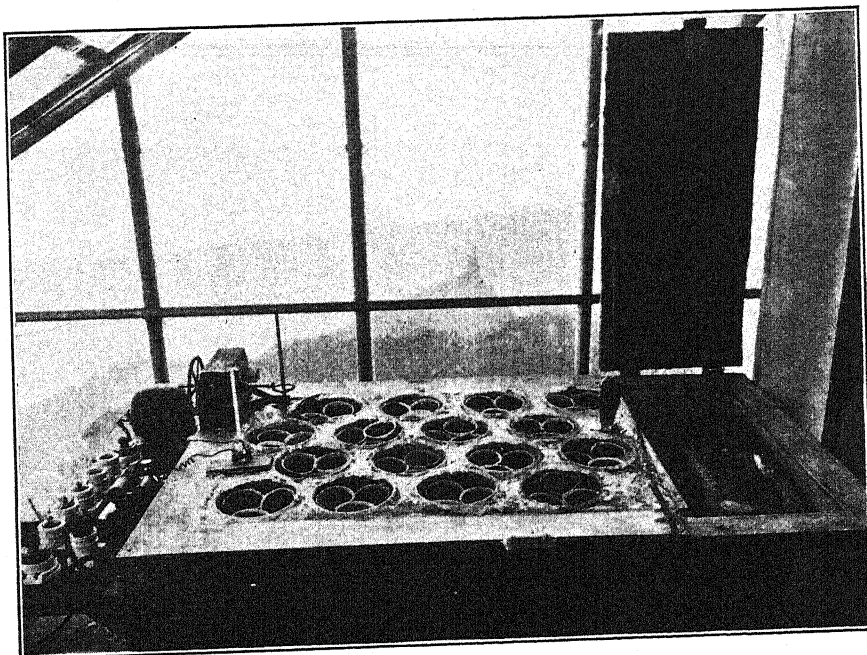


TEXT FIG. 3. Constant-temperature tank. 1, thermo-regulator; 2, 3, wires to relay; 4, metal cover; 5, electric switch; 6, relay; 7, shelf to support motor; 8, wooden box; 9, hair-felt lining; 10, outer tank; 11, air chamber; 12, drain cock; 13, supports for inner tank; 14, drain cock for inner tank; 15, shelf for ice; 16, door to ice chamber; 17, felt lining; 18, asbestos cover; 19, heater coil; 20, cable to electric switch; 21, battery jar; 22, germination chamber in place; 23, moist sand around germination chamber; 24, shelf for battery jars; 25, support for shelf; 26, inner tank; 27, ice chamber; 28, water at constant temperature.

An electric heater coil was connected to a thermo-regulator through a telegraphic relay. With the aid of a stirring apparatus the temperature of



the water was controlled to the accuracy of  $0.1^{\circ}$  C. from any desired point. Battery jars two thirds full of moist sand were supported on a shelf of the inner chamber as shown in text figure 3. Around the jars a cover of  $1\frac{1}{2}$  inches of asbestos was closely fitted to prevent excessive radiation.



TEXT FIG. 4. Constant-temperature tank installed.

Preliminary tests demonstrated that the soil temperature could be more easily controlled if the air temperature of the tank room could be kept equal to, or below, the desired soil temperature. To meet this need a small addition was constructed at the end of one of the greenhouses in such a manner that the temperature of this outer room could be raised by opening the greenhouse door, thus permitting heat from the greenhouse to enter it. The air in this room may be cooled by closing the greenhouse doors and opening the ventilators. Low soil temperatures were run in the winter without the use of ice, but in the summer ice was necessary for low temperatures. When the drain pipe of the outer chamber is closed, a jacket of water at the temperature of melting ice is secured to maintain very low temperatures.

#### EXPERIMENTAL PROCEDURE AND RESULTS

From the numerous preliminary experiments of the season of 1921-1922, both in the greenhouse and in the field, it was found that barley seedlings germinated satisfactorily between pH values of 5 and 8, at moisture percentages between 30 and 60 percent of the water-holding capacity, and at temperatures between 5 and 30 degrees centigrade.



In order to test the effect of temperature, moisture, and acidity simultaneously, one variety of barley was subjected to two moistures at the following H-ion concentrations: pH 5, pH 6, pH 7, pH 7.8. Thus eight sets of plants (4 at each moisture content) were germinated in the temperature tanks at each of the following temperatures centigrade: 5, 10, 15, 20, 25, and 30.

Seed of the variety Hannchen, a distichon barley, was used throughout the experiment. This seed was selected from smut-free rod-row plantings of the previous season and stored in a dry, cool laboratory. When used the seed gave 98-100 percent germination.

The smut used was from a collection made in North Dakota in 1922. This collection contained smutted heads of several different varieties, one of which was a two-rowed variety identified from some partially smutted heads as Hannchen. Smutted heads of this variety were selected from the mixed collection and the smut was powdered by grinding it in a mortar. The smut spores gave over 75 percent germination at room temperature in 24 hours in water and bouillon.

Inoculation of the seed was made by placing sufficient seed for the experiment in a 500-ml. bottle and shaking thoroughly with an excess of the powdered smut until the spore load was as heavy as the seed would carry. Inoculation was made one month before the experiment was started.

Soil of the desired hydrogen-ion concentration was secured by taking a neutral top soil of a good, rich quality and making it acid by the addition of dilute sulphuric acid. After adding acid the soil was dried, thoroughly mixed, sifted, and again resoaked with dilute acid, dried, mixed, etc., until the desired pH reaction was reached. This was a very laborious, time-consuming process, taking more than two months to reach the desired concentration. Every precaution was taken to insure a soil thoroughly mixed and uniform throughout. It was reduced to the desired moisture content, based upon the moisture-holding capacity calculated upon the oven-dry weight (40, 41), and then stored in a closed galvanized-iron container until used. In addition to the two acid soils, a check of the original neutral soil was run, and of one made alkaline by the addition of finely powdered air-slaked lime. This latter gave a pH reaction of 7.8. The acidity of the soil was determined by the colorimetric method as outlined by Wherry (57), Clark (11), and others.

The author realizes the incomplete state of our knowledge in regard to soil acidity, and that soils in nature may show the same pH reactions from quite different causes. The method of using dilute sulphuric acid to adjust the soil reaction was, therefore, empirically chosen in this experiment. Its use was suggested by the work of Hopkins (22), who adjusted the soil acidity with dilute sulphuric acid for his study of hydrogen-ion concentration in its relation to wheat-scab infection.

Having learned from preliminary tests that several barley seeds could

be germinated in paraffined drinking cups and then separated upon transplanting to the bench without any apparent injury, several seeds were planted in each cup. In this way 64 seeds were planted in each set, making a total of 512 seeds at each temperature, except in the tank at 5° C., where 480 seeds were planted.

The seeds were planted one inch below the soil surface in paraffined drinking cups. These cups were then fitted into the moist sand in the partially immersed battery jars of the temperature tank, as shown in text figure 4. Loosely fitting glass covers over the battery jars prevented excessive evaporation and maintained a fairly constant moisture content. The moisture content was checked by weighing the cups before placing them in the tank and then reweighing them at intervals. It soon developed that loss of weight could be avoided at lower temperatures by a proper adjustment of the glass covers. These covers were suggested by the work of Brefeld (4), who found a moist chamber closed with glass plates satisfactory for infection by various cereal smuts.

The seedlings were removed from the tank only after the coleoptile had been broken and the first green leaf protruded as much as one centimeter above it. Beyond this stage it has been demonstrated (1, 47) that infection by this fungus does not take place. The young seedlings were then separated and planted in the greenhouse benches and suitable conditions for barley growth were maintained.

The moisture content of the bench soil was maintained as nearly as possible between 50 percent and 60 percent of the moisture-holding capacity, which was shown by Sorauer (49) to be the most favorable moisture for this crop. The temperature of the greenhouse benches was kept between 55° and 70° F., as recommended by Hutcheson and Quantz (24) for barley.

#### Series I. Constant-temperature Experiments

The first of the series was planted in the tanks December 18, 1922, and the last had been transplanted to the greenhouse benches February 13, 1923. The first plants began to head April 10, and all had headed by May 10. The results are recorded in table 2.

These data are summarized in text figures 5 and 6, each containing four graphs in which all conditions are the same, as heretofore described, except the one of pH value.

*Results at 5° C.* The low percentage of infection is striking in both moistures and for all four acidities when the germination temperature is kept at 5° C. At this temperature the plants began to emerge from the soil the 22nd day after planting, and all had been transferred to the greenhouse benches by the 28th day.

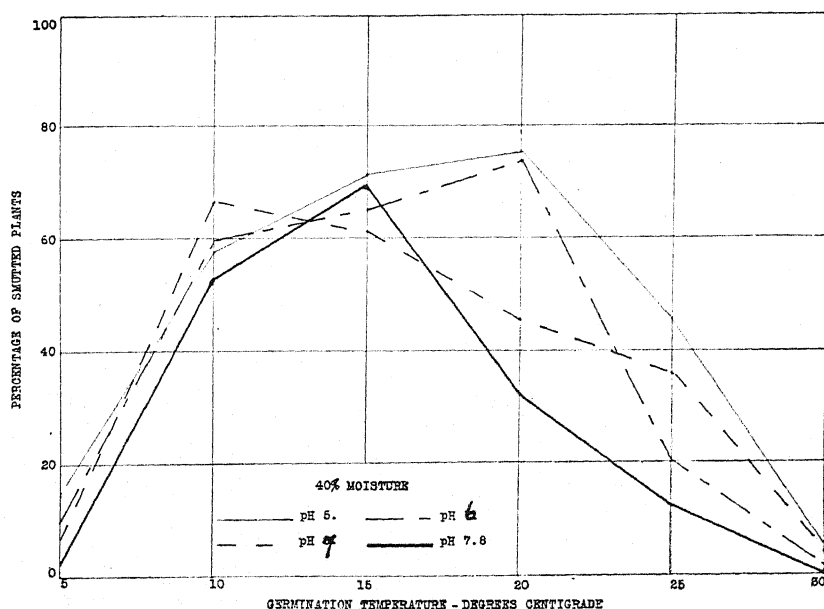
Total number of plants matured.....	463	
Total number of plants infected.....	<u>31</u>	
Percentage of infected plants.....		6.7%

TABLE 2. *Constant-temperature Experiments*

Germination Temperature	Moisture	pH	Plants				
			Total No.	No. Totally Smutted	No. Partially Smutted	Total No. Infected	Percent Infected
5° C.	40%	5	55	7	1	8	14.5
		6	59	5	1	6	10.1
		7	59	3	1	4	6.7
		7.8	60	1	0	1	1.6
	50%	5	61	5	0	5	8.2
		6	59	3	2	5	8.4
		7	53	2	0	2	3.8
		7.8	57	0	0	0	0
10° C.	40%	5	61	33	2	35	57.4
		6	62	34	3	37	59.7
		7	61	35	5	40	65.6
		7.8	62	32	1	33	53.2
	50%	5	62	48	3	51	82.3
		6	62	40	5	45	72.6
		7	61	29	2	31	50.8
		7.8	57	19	5	24	42.1
15° C.	40%	5	62	40	4	44	70.9
		6	60	33	6	39	65.0
		7	62	32	6	38	61.3
		7.8	62	40	3	43	69.3
	50%	5	62	34	4	38	61.3
		6	59	32	10	42	71.2
		7	63	33	3	36	57.1
		7.8	62	19	3	22	35.5
20° C.	40%	5	61	43	2	45	73.8
		6	55	35	5	40	72.7
		7	64	25	4	29	45.3
		7.8	60	16	3	19	31.7
	50%	5	60	45	5	50	83.3
		6	60	37	6	43	71.7
		7	62	20	1	21	33.9
		7.8	64	10	5	15	23.4
25° C.	40%	5	59	26	1	27	45.7
		6	59	10	2	12	20.3
		7	62	16	6	22	35.5
		7.8	55	7	0	7	12.7
	50%	5	62	32	4	36	58.0
		6	58	26	3	29	50.0
		7	61	20	4	24	39.3
		7.8	63	13	1	14	22.2
30° C.	40%	5	64	3	0	3	4.7
		6	62	1	0	1	1.6
		7	58	2	0	2	3.4
		7.8	31	0	0	0	0
	50%	5	60	1	0	1	1.6
		6	59	5	0	5	8.5
		7	60	4	0	4	6.6
		7.8	57	1	0	1	1.7

*Results at 10° C.* A very marked increase in infection was secured at this temperature in every degree of pH in both moistures. In some cases, *i.e.*, pH 7 at 40 percent, pH 6 and pH 7.8 at 50 percent, the maximum for the series was reached at this temperature. The first plants began to emerge from the soil the 12th day after planting, and all had been transplanted to the bench on the 18th day.

Total number of plants matured.....488  
 Total number of plants infected.....296  
 Percentage of infected plants..... 60.6%



TEXT FIG. 5. Graph showing effect of temperature and acidity upon percentage of plants infected at 40 percent soil moisture during germination.

*Results at 15° C.* The percentages remained high at this temperature throughout the entire series. The first plants appeared on the 7th day after planting, and all had been transplanted by the 11th day.

Total number of plants matured.....492  
 Total number of plants infected.....302  
 Percentage of infected plants..... 61.4%

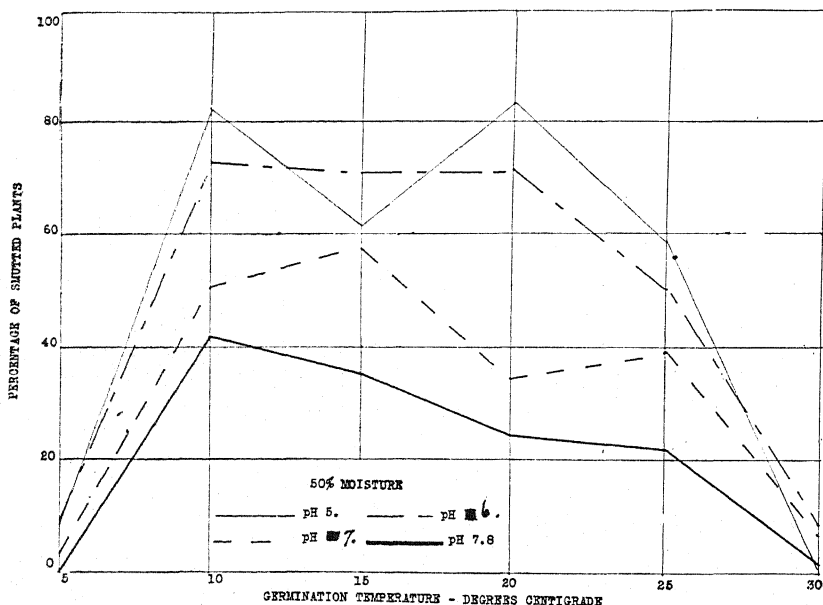
*Results at 20° C.* The highest percentages of this constant-temperature series were reached at this temperature, the strongly acid soil in both series giving the highest infection at this point. The first plants appeared above the soil surface on the 5th day, and all had been transplanted to the bench by the 9th day. The two acid soils at the low moisture were much slower than the other six sets, requiring three days longer to reach the proper size for transplanting.

Total number of plants matured.....	486
Total number of plants infected.....	262
Percentage of infected plants.....	53.9%

*Results at 25° C.* The falling off is consistent at this temperature, but the percentage of infection is still comparatively high. The plants in general made the most rapid growth of the entire series at this point. However, plants in the two acid soils lagged in both moistures, and the seedlings in the alkaline soil at the low moisture were slow in coming up. The first plants appeared the fourth day, and all had been transplanted on the 9th day.

Total number of plants matured.....	479
Total number of plants infected.....	171
Percentage of infected plants.....	35.7%

*Results at 30° C.* The percentages of infection approach very near to, or reach, the zero point at this temperature in all eight sets of the series. The high alkalinity at the low moisture seemed to have a deleterious effect upon the germination of the seeds, and the number of plants of this set at this high temperature was reduced to 31, none of which were infected. At this temperature the first plants began to appear on the 6th day, and all had been transplanted to the bench by the 14th day. The emergence of the plants was more irregular in this tank than in any of the others of the series. While it took longer for the plants to get above ground at the low temperatures, they emerged with remarkable regularity.



TEXT FIG. 6. Graph showing effect of temperature and acidity upon percentage of plants infected at 50 percent soil moisture during germination.

Total number of plants matured.....	451	
Total number of plants infected.....	<u>17</u>	
Percentage of infected plants.....		3.7%

At the higher moisture during germination the pH reaction is differential, the alkaline soil giving low infection throughout as compared to the neutral and acid soils. The more acid the soil, the more the smut percentages were increased at temperatures from 10° to 25° C. There is one irregularity, where the curve for pH 5 drops below that for pH 6 at 15° C. It is not clear at present why the percentages of infection in the acid soils at 15° C. should drop below those at 10° C. and 20° C. at this state of soil moisture.

The differences in the infection of the seedlings germinated in the acid and alkaline soils at the high moisture are very marked throughout the temperature ranges from 10°C. to 25° C., the percentage in the acid soil being almost double that in the alkaline soil in practically every case. The percentage of infection in neutral soil falls far below that in the acid soils, although it is somewhat higher than that in the alkaline soil. It should be noted that the acid soils gave higher infection at the higher moisture, but this is not true of the neutral field soil, run as a check, nor of the alkaline soil. In these latter soils the higher percentages of infection were secured at the lower moisture. Further experiments must be made before any general statement as to the relation of soil moisture to infection by this smut can be formulated.

#### Series II. The Influence of Varying the Temperature upon the Percentage of Infection

This series was carried out in the same general fashion as series I. Inoculated seeds, from the same lot as those of the previous series, were planted at both moistures and through the same pH ranges as in the constant-temperature experiments. These seeds were planted in a like manner in the temperature tank at 10° C. and held at this point for six days. At the end of the sixth day, when the coleoptile of the most advanced seedlings was just beginning to break away the outer hull, the temperature was gradually raised through a period of 36 hours until the soil in the cups had reached 25° C. The tank was gradually cooled for 12 hours to 15° C. and held there until the plants were large enough to be transplanted into the greenhouse bench. Only 48 seeds were grown in each set of this series, the result of which is given in table 3.

There are several points of interest in this series when compared with the constant temperatures of series I. The same material was used throughout, and the experiment was carried out in exactly the same way with the exception of the temperature change. Infection percentages are much higher in every set of this series than was reached at any of the temperatures of series I.

TABLE 3. *Effect of Varying the Temperature on Percentage of Infection of Hannchen Barley by Ustilago hordei*

Moisture	pH	Total No. Plants	No. Totally Smutted	No. Partially Smutted	Total No. Infected	Percent Infected
40%	5	46	39	1	40	86.9
	6	47	41	0	41	87.2
	7	48	45	1	46	95.8
	7.8	47	38	3	41	87.2
50%	5	48	46	1	47	97.9
	6	46	44	1	45	97.8
	7	42	35	3	38	90.4
	7.8	45	27	5	32	71.1

### Series III. The Effect of the Vigor of the Host After Germination upon the Development of Covered Smut of Barley

Hannchen barley inoculated with smut spores, as in series I and II, was used in this series. The experiment was carried out in a similar manner to that followed in series II. The seeds were germinated in the neutral soil in the temperature tank at variable temperatures simultaneously with those of series II, and then the plants were separated and transplanted in the greenhouse bench; one half of the plants were planted in a neutral soil, and the other half in an acid soil testing pH 4.6. That is, all plants were subjected to the same soil, temperature, moisture, and acidity conditions during germination, and then were separated at random when transplanted.

The acid soil used in this series, after the germination of the seeds, was from a lot of top soil, purchased commercially, and had not received the treatment with dilute sulphuric acid as described heretofore.

The plants were planted very closely in the bench because of lack of space, and this reduced the amount of tillering. However, each set of plants had an equal amount of room, and, since four of the acid set were lost while only one of the neutral set failed to mature, the acid series actually had more room in proportion to the number of plants than the neutral set.

The results of this experiment are given in table 4.

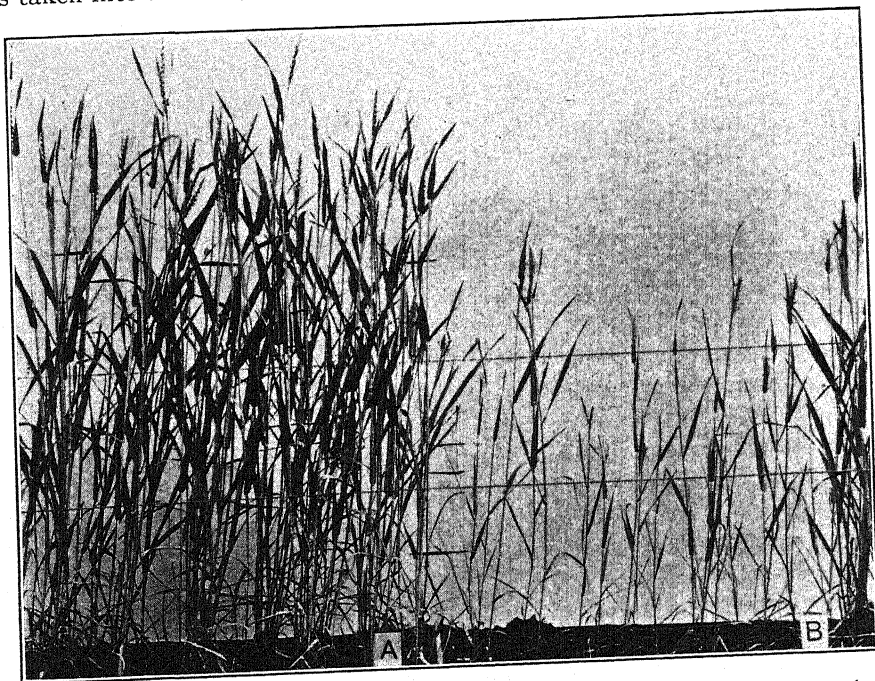
TABLE 4. *Effect of Vigor of the Host after Germination upon the Development of Covered Smut of Barley*

"Acidity" of Soil Used after Germination	Number of Plants			Number of Heads			No. Stalks per Plant Produced	Average Height Culms (cm.)
	Total	No. Inf.	Percent Inf.	Total	No. Inf.	Percent Inf.		
pH 4.6 . . . .	36	34	94.4	47	42	89.4	1.3	45
pH 7. . . . .	39	35	89.7	103	99	96.1	2.6	74

The plants matured in the acid soil were very much weaker, produced only one half as many tillers, and were a little more than one half the height of the plants grown in the neutral soil (text fig. 7 and table 4).



The percentages of infection are very high in each case and differ by very small amounts. When the small number of plants in the experiment is taken into account, this slight difference is not significant. So far as the



TEXT FIG. 7. Photograph showing effect of the vigor of the host upon the development of covered smut of barley (see text). *A*. Matured in a neutral soil, pH 7. *B*. Matured in an acid soil, pH 4.6.

evidence goes, the vigor of the host does not enable a susceptible variety of barley to escape the disease, once it is infected, nor, on the other hand, does the stunting of the plants to the degree observed in this experiment eliminate the smut.

#### Physiological Specialization in Covered Smut of Barley

After two seasons of intensive work upon this problem, involving the handling of about 100,000 plants in greenhouse and field experiments, it was discovered that the covered-smut spores secured from different geographical regions and from different varieties do not infect the same variety equally, even when the seed carries the maximum load of viable spores and is germinated and grown under the same temperature and moisture conditions. For example, Hannchen barley was inoculated with spores from Texas winter barley collected in Virginia and planted in the field. A total of 807 plants were produced, 9 of which were infected, or 1.1 percent. A similar field planting was made with the same variety inoculated with spores

from Hannchen barley collected in North Dakota. A total of 655 plants were produced, 166 of which were infected, or 25.3 percent.

That there are specialized races of this smut was suspected when the writer received a large collection of smut from North Dakota upon barley varieties which had given no smut in the numerous trials at the Brooklyn Botanic Garden. This material was received in July, 1922, and a test of this hypothesis was immediately devised, but the season was so far advanced that the plants did not head and no results were secured. During the winter comparative tests were made in which five different varieties of barley were inoculated with smut from three different regions of North America. The smut collected in Brooklyn was a mixture from occasional smutted heads appearing in a few of the 114 varieties of spring barley under test which had been heavily inoculated with covered smut obtained from Texas winter barley grown in Virginia.

Altona and Hannchen are awned, two-rowed spring barleys. Han River, Texas winter, and Hansee Hull-less are awned, six-rowed winter barleys.

The seeds were inoculated with the maximum spore load and germinated in the temperature tanks in a neutral soil with 40 percent of moisture and at a temperature of 20° C. After they had reached the proper size they were transplanted to the greenhouse benches as in the previous experiments. It should be noted that all three collections of smut were from the 1922 crop and gave 75 percent or more germination at room temperature in both water and bouillon.

The results of this experiment are given in table 5.

TABLE 5. *Experiment to Determine the Effect of Using Smut from Various Sources upon Infection of Certain Varieties of Barley*

Seed No.	Variety	Smut from Hannchen			Smut from California			Smut from Virginia		
		Total Plants	Plants Inf.	Percent Inf.	Total Plants	Plants Inf.	Percent Inf.	No. Plants	Plants Inf.	Percent Inf.
46	Altona.....	64	10	15.5	60	12	20.	63	6	9.3
101	Hannchen.....	44	20	45.5	42	0	0.	43	0	0
133	Hansee Hull-less	41	3	7.3	41	1	2.4	17	9	52.9
136	Han River.....	37	3	8.1	41	5	12.2	Did not head		
143	Texas Winter...	46	0	0	36	0	0.	4	1	25.0

This experiment was an attempt to eliminate the local soil variations which might account for some of the differences in infection secured in field plantings. The temperature (20° C.) was the same throughout the germination stages for all plantings, and the plants were transferred to the greenhouse bench at the same time. The soil was a thoroughly mixed field soil, neutral in reaction, its moisture content being brought up to 40

percent of the water-holding capacity. Great precaution was thus taken to see that all the collections of the smut had equal chance to infect the different varieties of barley.

Forty-five and one half percent of the plants of Hannchen barley (S. N. 101) were smutted when the inoculum came from that variety, but none were infected when the California or Virginia collections of the fungus were used. On the other hand, the California-collected smut produced 20 percent infection in the variety Altona (S. N. 46), and the Virginia smut produced 52.9 percent smut in the variety Hansee Hull-less (S. N. 133) and 25 percent in Texas winter (S. N. 143). Therefore the lack of infection of Hannchen by these latter two collections of smut is not a matter of the viability of the spores. Nor can the low infection of Hansee Hull-less and the lack of infection of Texas winter by the spores from Hannchen barley be thus explained. I can explain this result in no other way than upon the assumption that we are here dealing with a *physiological specialization* of the fungus.

### Field Experiments

In extensive field experiments, comparative lots of seed of 114 varieties of spring barley were inoculated with smut from Brooklyn, California, and North Dakota. In the latter case the smut was taken from heads of a hooded barley in the Dakota collection and not from Hannchen. However, some of the Hannchen barley seed, inoculated with smut from the Hannchen variety of the Dakota collection, had been planted. In fact, the seed had been taken from the same inoculated lot used in the experiments of series I.

This experiment was made under field conditions, the soil being about neutral in reaction. The soil moisture during the germination period ranged between 50 and 60 percent of the moisture-holding capacity, and the soil temperature varied from 3° C. to 23° C. as recorded by the soil thermograph.

Table 6 summarizes the results of these experiments upon the seven varieties which proved to be susceptible to the various collections of smut under the field conditions. Owing to a lack of smut material from Brooklyn (which originally came from Virginia), only a limited number of varieties were inoculated with this smut. These data are supplemented by reports from the 1922 crop. While the 1922 results are not exactly comparable with the 1923 results, they show what the same varieties have done when inoculated with smut spores from the same original source and grown in the field.

Nepal and Aegiceros are both naked hooded barleys, and both proved to be susceptible to the smut collected upon the hooded barley but were entirely free from smut when inoculated with the spores collected in California and Virginia. Furthermore, the varieties Nutans, Summit, and Surprise were more heavily infected with the smut from California than with that from the other sources.

TABLE 6. Field Tests Showing the Reaction of Seven Varieties of Barley to Four Collections of the Covered-smut Fungus.

Seed No.	Variety	Dakota Smut Smut on Hammchen			Dakota Smut Orig. Smut on <i>nuda</i>			California Smut Smut on Unknown Variety			Virginia Smut Smut on Texas Winter		
		Total Plants	No. Inf.	Percent Inf.	Total Plants	No. Inf.	Percent Inf.	Total Plants	No. Inf.	Percent Inf.	Total Plants	No. Inf.	Percent Inf.
66	Nepal		No test		53	26	49.	29	0	0	100/120	0	0
77	Aegiceros		No test		66	9	13.7	64	0	0	100/120	0	0
87	Aegiceros		No test		62	12	19.4	20	0	0	100/120	0	0
101	Hannchen		No test		108	2	1.8	100	1	1	96	1	1.0
163	Nutans	68	49	72.0	91	3	3.3	73	12	16.4	100/120	4	4
181	Summit		No test		52	4	7.7	56	19	33.9	120	0	0
182	Surprise		No test		81	5	6.1	63	9	14.3	100/120	0	0

Here again Hannchen produced 72 percent of smut when inoculated with spores from Hannchen, but gave only 1 percent and 1.8 percent smutted plants when inoculated with spores from the other sources, thus confirming the results of the controlled experiments performed in the greenhouse (table 5). There seems no other logical explanation of this result than that we are dealing with specialized races of the fungous parasite.

By way of explanation it should be stated that the results of the greenhouse experiments reported in the previous table (table 5) were not known until more than a month after these field plantings had been made. Otherwise more extensive cross-inoculation experiments would have been made with varieties upon which the various collections of smut gave high infection.

Therefore these results (tables 5 and 6) are being submitted, not as a complete statement as to these specialized forms, but to indicate the nature of the evidence from which the writer concludes that *Ustilago hordei* (Jens.) Kell. & Sw. is composed of several physiological races.

#### DISCUSSION AND CONCLUSIONS

##### Physiological Specialization

The careful study of environmental factors under controlled conditions, results of which are reported in tables 2, 3, 4, and 5, shows that high percentages of infection can be gotten over wide ranges of soil temperature and acidity and at moistures well within those usually existing when barley is planted in the field. It is evident, therefore, that the erratic infection results secured by the writer and other workers (10, 48) cannot be explained wholly by the limitations of environment as expressed in any of the soil factors here investigated. Furthermore, as is well brought out in table 6, no unusual soil conditions are necessary in order to secure high infections. The one requirement necessary to get the large percentages of infection in Hannchen and Nepal barley, reported in this table, was to dust the seed with the smut which had been collected on these respective varieties.

My early attempts to obtain infection by this method of dusting the seed with spores and planting it in the field failed to give as high infections as were found in the grain fields of the farmers (48). Broili (10) also reports that he was unable to secure satisfactory infection of barley with covered smut imported from foreign localities. This experience led him to state that he would use only native forms of the fungus in the future, but no proof is given that there exist what he terms *bodenständige* or *einheimische* races of this smut.

Tisdale (48), in Virginia, and Mackie (48), in California, likewise were unable to determine the resistance of barley varieties to covered smut because of a lack of satisfactory infection. Mackie (48) suggests that soil and climatic conditions may influence to a marked extent the amount of covered smut in barley.

The results of my experiments, however, indicate that the biologic form of the smut used with a particular variety of barley has far more to do with securing high infection than do the soil conditions, although the amount of infection may be varied greatly by certain rather extreme combinations of external soil influences.

Up to this time physiological specialization has been assumed to be lacking in the cereal smuts. Gaines (18) gives expression to the commonly accepted view by stating that "bunt, in common with other smuts, apparently consists of but a single biologic race." Upon what grounds such views have been held is not clear, especially in light of the facts brought together by Reed (42) which point to the general occurrence of specialized races in parasitic fungi. Recently Zillig (59) has brought forth proof of the existence of several biologic forms in the anther smut (*Ustilago violacea* (Pers.) Fcl.)

In attempting to produce smut-resistant varieties of the cereals, the breeder is at once confronted with the problem of the stability of the fungus. If the parasite is made up of numerous races, then the problem of resistance must be studied from the standpoint of its particular *biologic* forms. This, in itself, greatly complicates the problem. But the question of the stability of these forms, that of their method of origin, and the possibility of new ones arising which may be able to infect varieties resistant to existing races, are matters of great scientific and practical interest.

#### Soil Factors Influencing Infection

The studies of soil factors influencing infection here reported apply to the race of the smut on Hannchen barley as herein differentiated. Whether these various environmental relations hold for other races of the covered-smut fungus can be determined only by further experiment. Likewise, the host relationships of the various possible specialized races require extensive studies over a period of time before their distribution and importance can be determined.

A separate analysis of each individual factor in smut-production is impossible. To maintain all factors constant except the one under consideration has been the aim in these experiments. It is obvious that this has not been, and can not be, attained except in a relative degree. These studies of the influences involved in infection by this smut have emphasized the inseparable connection of all factors concerned. The final amount of disease appearing is due to the interaction of a multitude of factors, only a few of which have been singled out for this study.

However, by selecting such major influences as temperature, moisture, soil reaction, etc., and making an analytical study of their nature, it has been possible to arrive at a general view of their relative importance. Other factors might be similarly studied, and no doubt each would be found to have some effect upon the final amount of disease appearing in the plants.



*Temperature Relations.* High percentages of infection were produced over a wide range in temperature. It would be very difficult for a variety of barley susceptible to this smut to escape high infection in field planting if the spores are present. The amount of infection is influenced unfavorably somewhat by the relatively low soil temperatures at the time of planting spring barley, and this may account for the fact that covered smut is much less a problem in connection with spring than with winter barley. It is clear from the results secured in the higher soil moisture (text fig. 6) that the optimum temperature for infection is dependent upon, and can be stated only in relation to, the other limiting factors.

The variation in temperature tested was found more favorable to, and gave higher infection than, any of the constant temperatures used (compare tables 2 and 3). This sort of variation in temperature is of exactly the type met in the field, and future studies might well include experiments to determine the influence of varying the temperature, under controlled conditions. By such studies a nearer approach to the natural conditions of infection could be made than is secured in the constant temperatures now so much in use in soil-infection studies.

*Moisture and Acidity Relations.* An increase in the moisture gave, in general, an increase in infection in the two acid soils. It would be interesting to know whether the low-lying river- and lake-bottom lands of California, mentioned by Mackie (48) as having more severely infected barley during wet years, have acid soils. In both degrees of moisture used, the acid soils gave high infections at temperatures ranging from 10 to 25 degrees centigrade. The pH reaction is differential at the higher moisture throughout this same range of temperature.

The writer is indebted to Dr. George M. Reed, Curator, Brooklyn Botanic Garden, for pointing out this problem and rendering much assistance and advice during the progress of the work; and to Prof. R. A. Harper for valuable suggestions and criticism during the progress of the experiments and the preparation of the manuscript.

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## DESCRIPTION OF PLATES

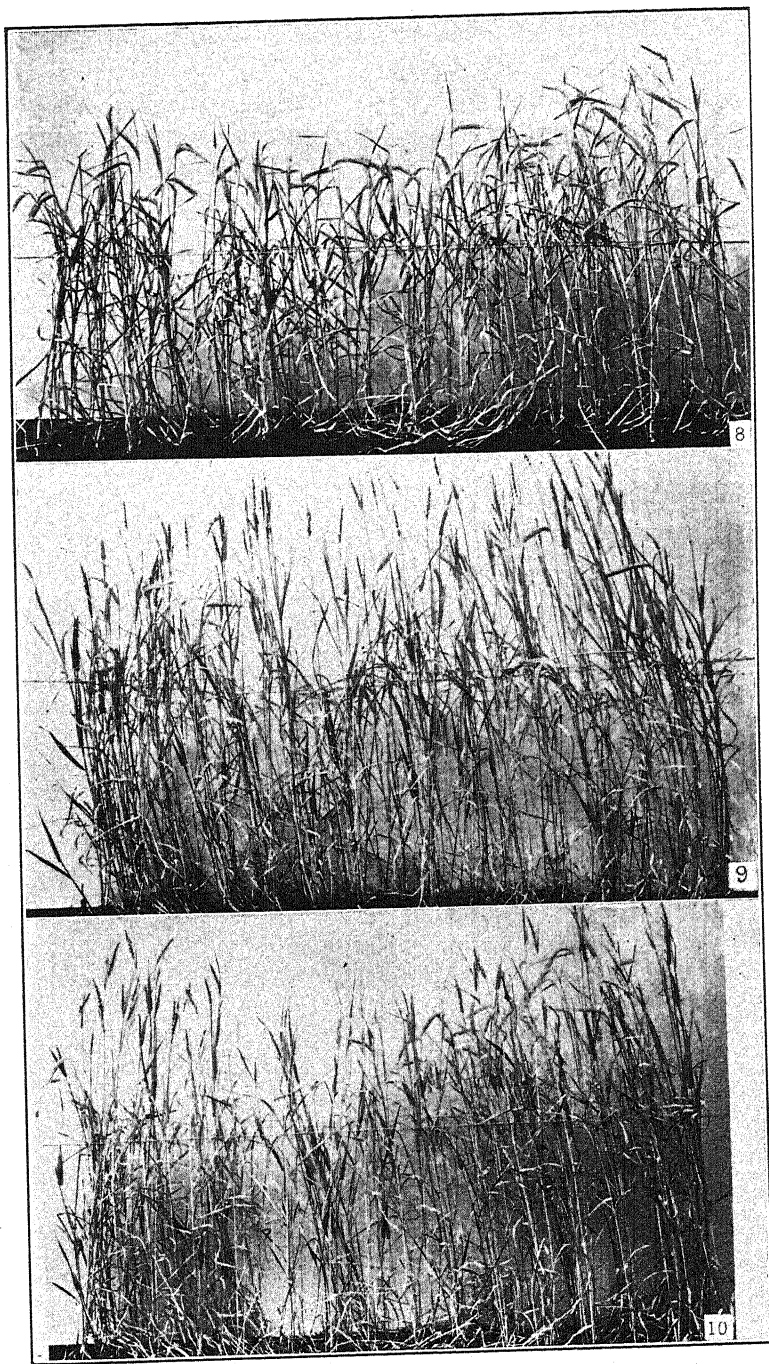
All figures are of Hannchen barley germinated in neutral soil at 40 percent moisture (see text).

## PLATE VII

- FIG. 8. Germinated at 5° C. 6.7 percent of plants infected.  
FIG. 9. Germinated at 10° C. 65.6 percent of plants infected.  
FIG. 10. Germinated at 15° C. 61.3 percent of plants infected.

## PLATE VIII

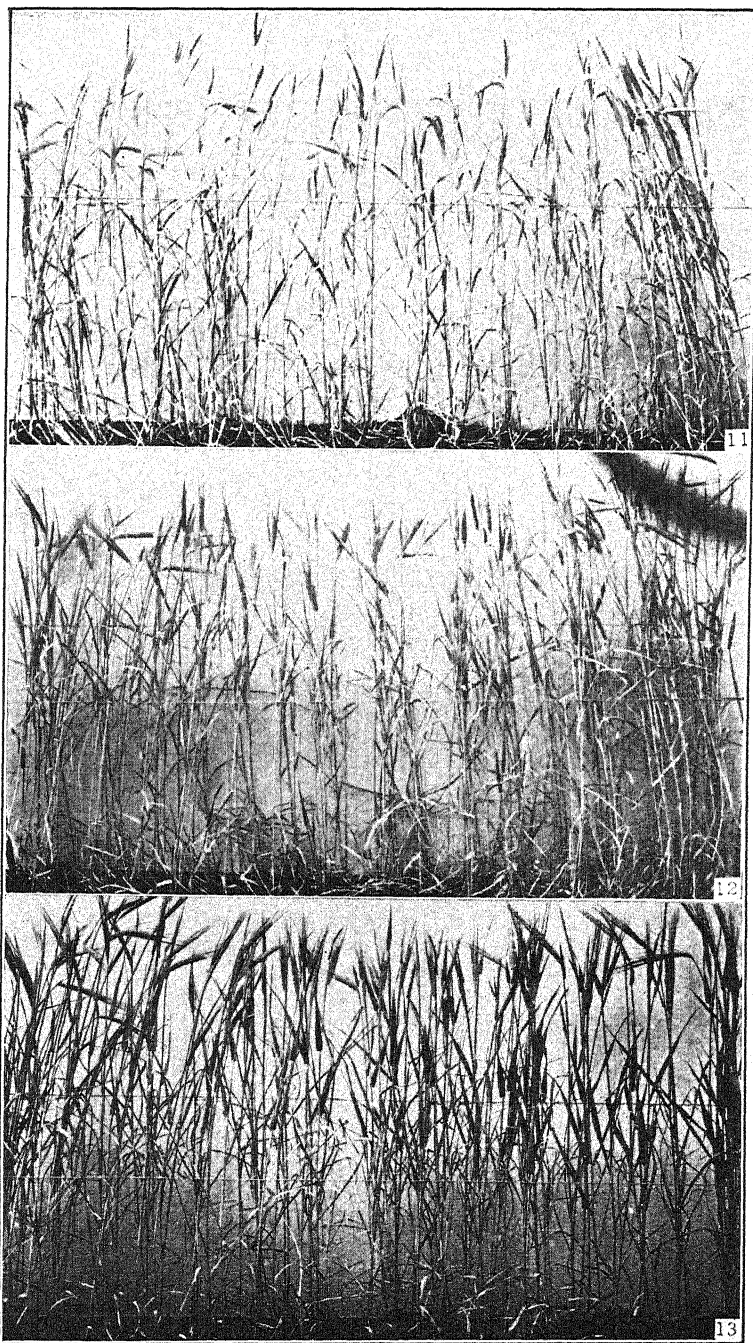
- FIG. 11. Germinated at 20° C. 45.3 percent of plants infected.  
FIG. 12. Germinated at 25° C. 35.5 percent of plants infected.  
FIG. 13. Germinated at 30° C. 3.4 percent of plants infected.



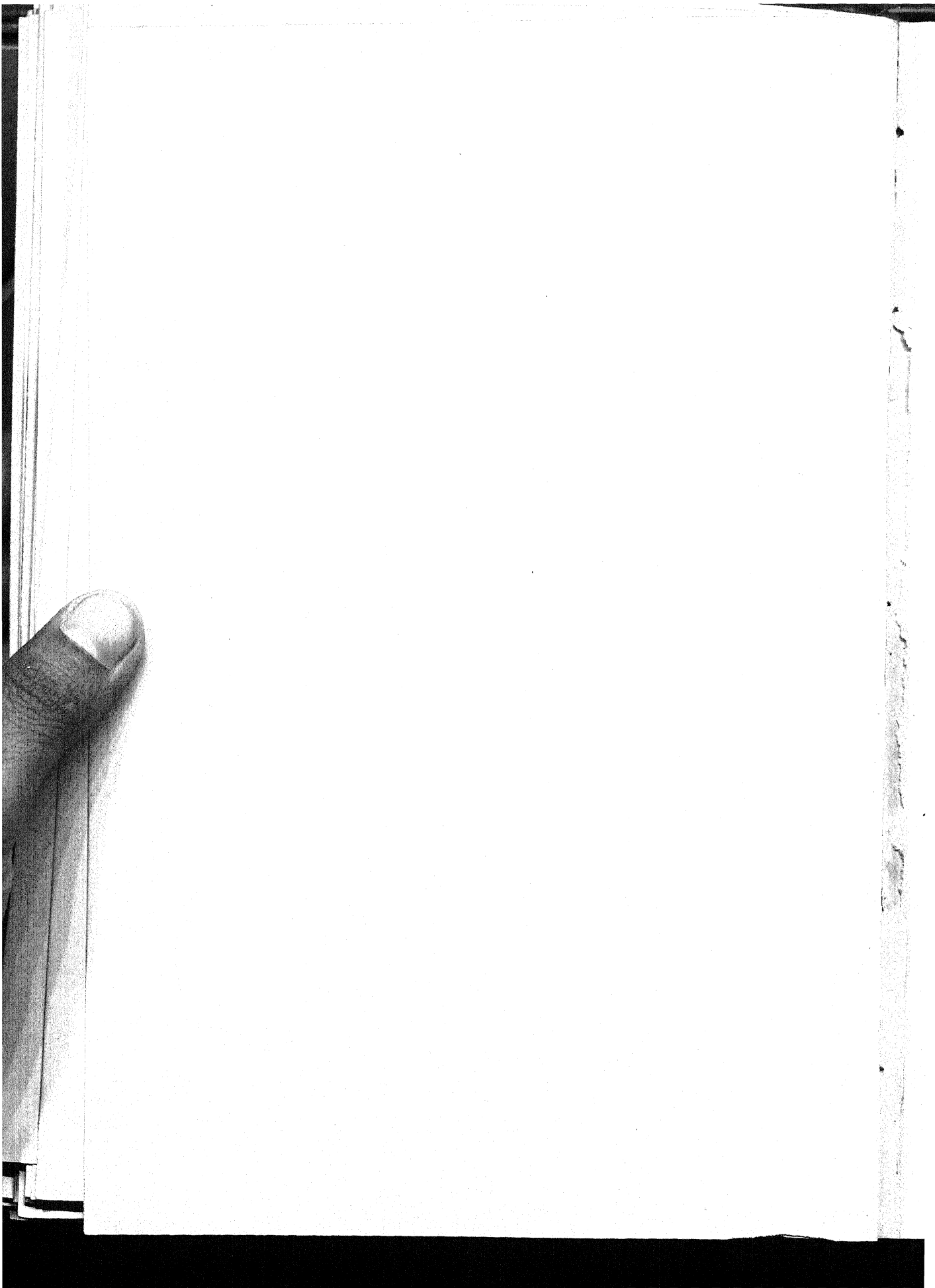
FARIS: INFECTION OF HORDEUM







FARIS: INFECTION OF HORDEUM





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## SOME RELATIONS OF *FUSARIUM LINI* AND POTASSIUM CYANIDE

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Since there is in the young flax plant a characteristic glucoside, linamarin, which splits into hydrocyanic acid and glucose, it seemed worth while to determine the reaction of *Fusarium lini*, the pathogen of the flax-wilt disease, toward several cyanogen compounds. The well known action of potassium cyanide on animals suggested that it would be well to test its effects upon the fungus. As early as 1875 it was shown (1) that hydrogen cyanide destroys the vitality of yeast, but little work seems to have been done in studying its relations to other fungi. Stevens (2) reported upon some work and called attention to the comparatively innocuous character of potassium cyanide in killing fungous spores. The splitting of several glucosides by enzymic action, including some cyanogenetic glucosides, has been described (3) for a few of the molds, especially the genus *Aspergillus*.

The immediate objectives in the experimental work here reported were to test the toxic effects of potassium cyanide upon *Fusarium lini* and to determine any possible stimulation of growth caused by it. The cultures were made in petri dishes, the synthetic agar culture having been treated with varying strengths of potassium cyanide. These were then inoculated with *Fusarium lini* in the center of each dish. A volume-molecular solution of KCN was made and diluted to the following strengths in the culture medium: 1/1000 M; 5/1000 M; 1/100 M; 2/100 M; 3/100 M; and so forth to 1/10 M. The record of growth was kept by measuring in millimeters the total diameter of the colony resulting from the inoculation in the center. The results are given in table 1, taking the average diameter of three cultures for each concentration, except in the cases indicated by stars, where only two are averaged.

TABLE 1. Figures Indicate Diameters in mm.

Hours after Inoculation	No KCN	.001 M KCN	.005 M KCN	.01 M KCN	.02 M KCN	Higher Concent.
46.....	18 mm*	13.5	14.3	10.7—	5.7—	None
77.....	32.2*	27.7—	24.8	18.7—	10.7—	"
125.....	53.6*	49.7	45.5*	35.2	25.3	"
151.....	65.0*	61.7	56.5*	48.9	36.1	"
16 days....	All plates completely covered with <i>F. lini</i>					

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It is evident that the first effect upon the fungus is to depress its growth in proportion (roughly) to the concentration of the KCN. The table of percentages of total growth, based upon the check plates as 100 percent growth, shows that none of the cultures containing the cyanide came closer than about 95 percent of the amount of growth shown by the checks, and ran as low as 31.6 percent in the cultures having the greatest concentration of cyanide and incubated for only 46 hours.

TABLE 2. *Percentages of Total Growth*

Hours after Inoculation	No KCN	.001 M KCN	.005 M KCN	.01 M KCN	.02 M KCN
46.....	100	75	79	59.4	31.6
77.....	100	86	77	58.0	33.2
125.....	100	92.7	84.8	65.6	47.2
151.....	100	94.9	86.9	75.2	55.6

If, however, the total percentage growth is calculated on the basis of the growth made during the first 46 hours for each concentration, it can be seen that the colonies with no KCN made a total growth of 261.1 percent of their growth during the first 46 hours. Those growing on the various KCN plates made higher percentage growths, increasing with the increasing KCN concentrations. Thus, to contrast with the 261.1 percent growth without KCN, the cultures on the .02 M KCN made a growth of 533.3 percent of their initial growth.

TABLE 3. *Total Percentage of Growth, Based upon the Initial Growth during the First 46 Hours, in each Concentration of KCN*

Hours after Inoculation	No KCN	.001 M KCN	.005 M KCN	.01 M KCN	.02 M KCN
46.....	0	0	0	0	0
77.....	78.8	105.1	73.4	74.7	87.7
125.....	197.7	268.1	218.1	228.9	343.8
151.....	261.1	357	295.1	357	533.3

The data may be still further analyzed by calculating the average *hourly* percentage growth on the basis of the initial growth for each concentration (table 4). Thus, in the cultures having the .02 M KCN, the colony had, at the end of 77 hours of growth, increased 87.7 percent over the growth attained in the first 46 hours, which makes 1.13 percent increase per hour for each of the 77 hours. The full table shows that in all concentrations of KCN this average percentage increased up to the end of the 151 hours of growth, while in the check plates, having no KCN, it increased very little.

The reason for this becomes clear when we compare the hourly percentages of increase during successive periods of growth (table 5). Thus, in the .02 M cultures the hourly percentage during the period of growth from the 46th to the 77th hours was 2.83 percent, while between the 125th and 151st hours it had mounted to 7.288 percent. Where there was no KCN the

rate was practically uniform throughout the whole 151 hours of growth, varying only from 2.44 percent to 2.54 percent. If we compare the 7.288 percent hourly average for the .02 M cultures with the 2.44 percent hourly average for the check cultures, it is evident that, while the KCN depresses the total growth, as shown by the first table, the rate of growth of the fungus is

TABLE 4. *Average Percentage Rate of Growth per Hour, on the Basis of Growth Made during the First 46 Hours, for each Concentration of KCN*

Hours after Inoculation	No KCN	.001 M KCN	.005 M KCN	.01 M KCN	.02 M KCN
46.....					
77.....	1.023	1.365	0.953	0.97	1.138
125.....	1.581	2.144	1.744	1.831	2.750
151.....	1.728	2.364	1.954	2.364	3.53

much greater during the later stages of incubation. This latter statement is true also of the .01 M concentration. The .001 M concentration was more like the check cultures in that the rate of growth was approximately uniform, but it was consistently higher. The greatest rate of growth in the whole series was in the highest concentration of KCN during the last stage of growth, that is, from the 125th to the 151st hour. This is especially interesting since one would naturally expect that the check cultures, having a better start, would increase at a greater rate. It is clear from the calculations in table 5 that the rate in all KCN concentrations was greater after the first slow period of growth than in the cultures free from the cyanide.

TABLE 5. *Change of Percentage Rate of Growth per Hour on same Basis as Table 4.*

Time of Growth	No KCN	.001 M KCN	.005 M KCN	.01 M KCN	.02 M KCN
31 hours.....	2.54	3.387	2.368	2.41	2.83—
48 hours.....	2.46	3.40	3.00	3.212	5.33
26 hours.....	2.44	3.419	2.96	4.93—	7.288

Whether these results are due to a stimulative effect of the KCN remains to be proven, although it seems very probable. It would seem also that the fungus is at first injured by the cyanide, but later becomes, not only habituated to it, but even benefited, at least in vegetative growth, by its presence. As shown in table 1, concentrations of potassium cyanide of .03 M and higher entirely prevented the development of *Fusarium lini* under the conditions of the experiment.

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## THE PHYSIOLOGY OF POLLEN

### I. THE REQUIREMENTS FOR GROWTH<sup>1</sup>

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Following the demonstration of sexuality in the higher plants by Cameraarius in 1694 and his recognition of the fact that in some way the pollen serves as the male element, a period of over a century elapsed before any definite idea was gained as to just how the pollen functions in fecundation. Amici (1824),<sup>2</sup> while examining the papillar cells of the stigma of *Portulaca oleracea*, noticed by chance that one of the "hairs" among those he was examining terminated in a pollen grain. This was the discovery of the pollen tube. Six years later, the same author (Amici, 1830) observed and described the formation of pollen tubes on the stigmas of *Hibiscus* and the gourd, and traced them through the tissue of the style to the ovary. Amici recognized that the pollen tube as a definitely organized structure grows down through the style. He further saw that one pollen tube passes to each ovule.

Brongniart (1827) had contended that the fertilizing element is transported to the ovary in the interstices of the conducting tissue of the style, without making out, however, just how this is accomplished. Schleiden (1837) and Schacht (1851, 1858) ascribed to the pollen tube the major rôle in the formation of the embryo, regarding the ovule as merely serving to enclose the tip of the tube which itself became the embryo. The careful researches of Hofmeister (1849) finally settled the bitter contention that arose regarding the respective rôles of the pollen tube and ovule in embryogeny by demonstrating clearly that, as Amici had maintained, the embryo arises from an egg within the ovule and that the pollen tube serves to stimulate this egg to development. When Strasburger (1884) described fertilization in the higher plants as consisting essentially of the union of the egg nucleus with a nucleus brought in by the pollen tube, the real significance of the pollen-tube growth in the style was first clearly appreciated. The rôle of the second male nucleus brought in by the pollen tube was cleared up when Nawaschin (1899) and Guignard (1899) independently discovered its fusion with two polar nuclei to form the primary endosperm nucleus.

In answer to his own question as to how tubes arising from the small pollen grains are able to traverse long styles, Amici (1830) advanced the

<sup>1</sup> Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

<sup>2</sup> References are to literature cited at the end of Part IV, to appear in a future issue of this JOURNAL.

view that the tubes derive nourishment from the styler tissue in their growth toward the ovary.

That pollen of one species might form tubes in the styles of very different forms was established by Strasburger (1886). In the styles of *Convallaria latifolia*, for example, it was found that pollen from *Fritillaria persica* formed tubes that grew the whole length of the style to the ovary. These failed, however, to enter the micropyle. Pollen of *Agapanthus*, a monocotyledon, germinated on the stigma, and grew readily in the styles, of *Nicotiana tabacum*, a dicotyledon, and *vice versa*. Examining the styles of many other species following foreign pollination, Strasburger noted that the phenomenon was of frequent occurrence and drew the important conclusion that pollen-tube growth could not be explained on the basis of sexual affinity. He suggested that, since pollen-tube growth in the style resembles so closely the penetration of the hyphae of parasitic plants into the tissues of their hosts, very probably a similar cause underlies both phenomena.

The early hybridists, notably Koelreuter (1761-1776) and Gärtner (1849), encountered a condition in certain plants whereby seed either failed to set or set incompletely after self-pollination, in spite of the fact that the pollen and egg cells themselves were normal as demonstrated by their ability to function completely in certain cross-pollinations. Among animals, Castle (1896) demonstrated that the hermaphroditic tunicate *Ciona intestinalis* exhibits a similar condition. This phenomenon, in which fertilization of the eggs by the male gametes of the same individual is impossible or at best irregular, is designated as *self-sterility*. It has recently been shown by East and Park (1917) that cross-sterility identical in nature with self-sterility occurs. While the number of cases in which self-sterility has actually been demonstrated is not large, East and Park contend with good reason that it is possibly a widespread phenomenon. Knuth (1906) has compiled a list of cases, the majority of them well founded, indicating that among the angiosperms the condition is rather generally distributed.

It was early shown by Scott (1865) and by Fritz Müller (1868) that pollen tubes are produced freely in the styles of self-sterile plants after selfing. Jost (1907) assigned as the immediate cause of self-sterility the failure of the pollen tubes after an incompatible pollination to grow rapidly enough to reach the ovary before the flower falls. Martin (1913), working with *Trifolium pratense*, a self-sterile form, found that the difference between self- and cross-pollinations was one of rate of growth. East and Park (1917), in their studies on *Nicotiana*, have secured abundant evidence to show that, following incompatible combinations, the rate of pollen-tube growth in the style is lower than that attending fertile combinations.

In that class of self-sterile plants designated as *heterostyled*, Darwin (1877) has shown that the peculiar fertility relations obtaining between the different forms of a given species are related to the differences in length of

style and in size of pollen grains. In *Lythrum salicaria*, for example, each of the three forms, namely, the short-styled, the mid-styled, and the long-styled types, bears in its flowers two sets of stamens different in appearance and function. In this heterostyled species eighteen different pollinations are possible only six of which result in complete fertility. In a series of carefully executed experiments Darwin demonstrated that each form is sterile with pollen from its own two sets of stamens and fully fertile only with pollen from the set of stamens of corresponding length in each of the other two forms. That within the flowers of one and the same plant there are differentiated two kinds of pollen so strikingly unequal in their potency when combined with a plant of another form as to result in the production of a full complement of seed in the one case and to prove unfruitful in the other is indeed a phenomenon at once extraordinary and perplexing.

It is probable, however, that the immediate cause of unfruitfulness following illegitimate combinations among heterostyled species is not unlike that responsible in cases of ordinary self-sterility. Illegitimate matings are sometimes followed by the production of a few seeds that will produce normal plants. This fact, taken in conjunction with Darwin's observation that, following illegitimate pollination, pollen tubes are freely produced in the styles, supports the view that here too the impediment to fertility is entirely a physiological one.

It will be evident from the foregoing discussion that the general problem of pollen physiology is one of considerable biological interest. The simple mechanical methods by which pollen grains reach the stigma of the female sex apparatus have long been familiar facts. General agreement has been reached on the behavior of the male gamete in the presence of the egg itself. But as to the fundamental processes involved in the growth of the pollen tube with its cargo of nuclear material from stigma to ovules there is still wanting a comprehensive interpretation based upon a sufficient body of well ascertained facts.

The present studies were undertaken with the object in view of gathering some further evidence of the requirements for pollen-tube development and growth. The extent to which the known facts will permit of a general interpretation of the phenomenon will, we hope, be indicated in the succeeding pages.

#### THE CHEMICAL COMPOSITION OF POLLEN AND CONDUCTING TISSUE

Mangin (1889) distinguished two layers in the pollen-grain wall. The external cutinized layer he called the *exine*; the *intine* or internal layer was found to consist of cellulose on its outer side, associated with some pectic substance which he named *callose*. Biourge (1892) confirmed these observations and noted that early germination consists of an evagination of the intine.



Czapek (1913, 1920, 1921) has brought together the results of the earlier chemical analyses of pollen. The evidence these afford, taken in conjunction with that obtained through microchemical studies by Lidforss (1899 *b*), Molisch (1893), and other investigators, indicates that the pollen grains themselves, like the seed, are provided with a considerable amount of food material in the form of carbohydrates or fats. The nature of this reserve material in a given form, according to the views of Molisch (1893), Lidforss (1899 *b*), and Sterner (1913), is dependent to a certain extent upon age of pollen, seasonal conditions, and geographic distribution. Heyl (1917) observed three nuclei in the pollen grains of ragweed and estimated the nuclear volume to be about 20 percent of the protoplasmic substance. Chemical analyses of the entire grain showed 24.4 percent protein, 10.8 percent fat, 0.75 percent lecithin, 5.4 percent ash, 7.5 percent pentosans, 12.2 percent crude fiber, and small amounts of sucrose, glucose, and dextrin. On continuing his investigations, Heyl (1919) determined that the walls of the pollen grains in this form constitute 65 percent of the structure. The results of the detailed analysis Heyl has made of the protein extract of ragweed pollen are especially instructive. A considerable quantity of non-protein nitrogen was found. The aqueous extract contained 1.2–1.5 percent coagulable albumin and a mixture of proteoses amounting to 3.0 percent. Peptone was found, and the hexone bases arginine, histidine, and lysine were present in abundance, arginine being found in the smallest proportion. Only a minute quantity of organic phosphorus was present. Extracting with dilute alkalis, a glutelin amounting to 2.9 percent was obtained. Heyl and Hopkins (1920) have, on further investigation, found that the albumin present in ragweed pollen is a normal protein except that it lacks histidine and perhaps tryptophane. The percentage of amino-acids found by these authors in the ragweed-pollen proteins is as follows:

	Proteose 5/10-10/10*	Proteose Entire Fraction Dialysed	Glutelin		Albumin
Arginine.....	1.48	2.08	4.69	4.70	6.15
Histidine.....	0	0	1.69	2.30	0
Lysine.....	3.70	4.48	7.66	6.60	8.76
Tyrosine.....	.78	1.10		4.7	2.79 2.83
Tryptophane.....	0	0	0		0

\* Saturation of  $(\text{NH}_4)_2\text{SO}_4$ .

Glutelin, which is the predominant protein, has histidine present among the bases and is therefore more nearly complete. The authors conclude that in pollen we have a series that may represent three stages of protein development: Native compound proteose ( $N = 5.4$  percent)  $\rightarrow$  albumin ( $-$ histidine)  $\rightarrow$  glutelin. In the synthesis of protein in the growing pollen tube, Heyl and Hopkins have not considered the possibility that nitro-



genous compounds are derived from the stylar tissue also. Anderson and Kulp (1922) have made extensive analyses of maize pollen. The principal reserve materials in this form appear to be carbohydrates in the forms largely of starch and sucrose. The percentages of starch and sucrose in three different varieties are reported as follows:

	Yellow Dent (Leaming)	White Flint (Luce's Favorite)	Pop Corn
Starch.....	11.07	19.04	18.03
Sucrose.....	9.09	2.97	14.18

The difference between the total amount of starch and sugar in the pop-corn pollen and that found in the other two varieties is large and perhaps of some significance. The differences in relative proportion of sugar and starch may not be important, since these are readily transformed one to the other. An analysis of the ash of Leaming pollen showed that potassium and phosphorus predominate, there being 35.58 percent of the former element present and 18.92 percent of the latter. Only 1.02 percent of calcium was found and 4.60 percent of magnesium. It was calculated that there is 3.62 percent of lecithin present in maize pollen. While the reserve food materials constantly found in pollen may be sufficient for initial growth, it is inconceivable that they are adequate in amount for the production of tubes that reach a length in certain species equal to many hundred times the diameter of the pollen grain itself. In the course of its development the pollen tube must draw upon outside sources of nutritive material.

The rôle of the conducting tissue in nourishing the pollen tubes was first defined by Amici (1824), the discoverer of these structures. Dalmer (1880) quotes Behrens (1875) as concluding from the results of his microchemical studies that the slimy substances of the stigma contain amyloid materials, sugar, and probably other carbohydrates. The anatomical investigations of Capus (1878) and of Dalmer (1880) revealed that reserve food materials are freely distributed in the tissues leading to the ovules. Green (1894) found that in the style of the lily the epithelial cells lining the stylar canal and several layers beneath them are filled with minute granules of starch. The outer layers of the style are free from these. Starch is plentiful, too, in the outer soft tissues of the fibrovascular bundles, indicating a definite deposit or reserve store placed in the conducting tissue. Green concludes, from the fact that the deposit does not extend to the stigma, that the reserve material in the style is intended for the growth of the pollen tube after it has exhausted the special store in the pollen grain. In *Micrampelis*, Kirkwood (1906) found that the cells lining the stylar canal and covering the placental lobes are filled with starch. A similar condition was observed in *Melothria* and *Cyclanthera*. The conducting tissue in *Cucurbita pepo*, however, appeared to be quite devoid of starch.

This does not necessarily indicate, as Kirkwood has observed, that the style of the pumpkin entirely lacks carbohydrates. Here, as in the other cucurbits examined, the style may provide nutritive material to the growing pollen tube in the form, probably, of cane sugar. Martin (1913) found that the exposed portions of the papillae of the stigma of *Trifolium pratense* are rather heavily cutinized. No starches or sugars were found, but an oily emulsion such as he had identified in the pollen is present.

#### ENZYM RELATIONS

Intimately related to the phenomenon of food-storage and utilization is the occurrence of enzymes in the pollen and conducting tissues. Erlenmeyer (1874) found evidence of diastatic activity in pine pollen. Van Tieghem (1886) observed that, following the addition of pollen of *Narcissus*, *Crocus*, and some other species to a 10 percent solution of cane sugar, with or without an antiseptic, the cane sugar was inverted. When pollen grains of some species were germinated in the presence of starch paste, Strasburger (1886) found that the starch was gradually hydrolyzed to sugar, indicating the presence of diastase. Green (1891), in a series of carefully conducted tests, demonstrated that diastase occurs in the pollen of *Lilium*, *Helianthus*, *Antirrhinum*, and several other species. In a second paper, Green (1894) reports the results of more extensive experiments. He found that diastase and invertase are frequently present in resting pollen but that their distribution is irregular, some forms containing both, others only one of them. Neither a cytolyt nor a proteolyt was found. Green's results on the changes in the amounts of enzym present during germination and growth are instructive. *Lilium* and *Narcissus* were selected as yielding diastase and invertase respectively. Quantitative determinations of enzymic activity were made with each form both upon fresh pollen and after cultivation on sugar solutions. A striking increase in reducing power was obtained with the pollen of *Narcissus* following germination. In *Lilium* it was found that, after several hours' growth, the diastatic power had increased as much as five times. Only a single exception to these results was observed. In a trial in which *Lilium pardalinum* was used, there appeared to be a temporary diminution in the quantity of diastase at the onset of germination. Lack of material prevented the repetition of this experiment. Working with a sample of *Lilium* pollen which had entirely lost its power to germinate through age, it was found that the amount of diastase present was only one third that originally contained in the fresh material.

Continuing his work, Green found that the pollen of *Zamia Skinneri*, one of the Cycads, contains neither starch nor diastase in its resting condition but that both were formed when the pollen was cultivated in solutions of sucrose or glucose. In water cultures alone, neither starch nor enzym was formed.

During the growth of the tubes of *Lilium* on artificial cultures, Green

was enabled to observe directly the digestion of the starch carried over from the pollen grains. Shortly after germination the starch granules, previously staining blue with iodine, were observed to be changing color. As the tubes increased in length, the granules nearer the grain end still stained blue, while those studding the greater part of the tube were purple. Toward the tip of the tube the granules were distinctly reddish. As Green concluded, "the starch was evidently in process of digestion by the diastase, ministering to the great formation of cellulose composing the wall of the tube."

Rittinghaus (1887) observed that the pollen tubes of *Lychnis*, *Agrostemma*, *Phlox*, and some other forms in passing into the stigma exerted a solvent action on the cuticle. He suggested that this might be due to an enzym. Kamman (1904) found diastase, invertase, and a proteolytic enzym in rye pollen. In a later paper Kamman (1912) reports the presence of lipase also. Sandsten (1909) found diastase and invertase in the pollen and styles of a number of plants. In some species of *Cassia*, Herman Müller (1883) found two kinds of pollen which he termed *Befruchtungs-pollen* and *Beköstigungs-pollen* respectively. Only the former produces tubes under ordinary conditions. Tischler (1910) found that the "*Beköstigungs-pollen*" could be induced to germinate by adding saliva or diastase to the culture and attributed its sterility to a lack of this enzym.

Recently Paton (1921) has reported the results of extensive studies on the occurrence of enzymes in the pollen of 18 species of plants distributed among 14 genera in 9 families. Catalase, diastase, invertase, reductase, and pectinase were found in all; pepsin, trypsin, erepsin, and lipase were found in some but not in others; cytase was not demonstrated with certainty in any; zymase was doubtfully identified in apple pollen; tyrosinase and laccase appeared to be entirely absent. The wide occurrence of pectinase is interesting in view of the generally observed intercellular growth of pollen tubes in the style. The rôle of enzymes in the metabolism of the pollen tube is unquestionably of considerable significance. It is to be hoped that Paton's contribution on the occurrence of a variety of catalysts in pollen may lead to further investigation of the processes in which they are involved.

#### THE ARTIFICIAL CULTURE OF POLLEN

Since von Mohl (1834) observed that pollen of some species would form tubes readily in moist air, numerous attempts have been made to cultivate it artificially. It has been found that different species vary considerably in the ease with which their pollen may be germinated *in vitro*. In some cases tubes may be induced to form under a rather wide variety of conditions, in others the requirements for germination are quite exact, and with not a few species the methods thus far devised have given only negative results. Even in those cases in which germination of the pollen is

possible, the growth subsequently obtained is, except in a few forms, almost negligible in amount when compared with that occurring normally in the style of the plant.

In spite of the varying degrees of success that have attended the attempts to cultivate pollen *in vitro*, the results that have been obtained with some forms show it to be a logical and promising method of attack upon the problems of pollen physiology. The seemingly inordinate variability sometimes encountered should not be permitted to obscure the results of experiments where regularity does prevail. The divergent results frequently obtained in artificial cultures of pollen are not, in the author's opinion, to be attributed entirely to the basic differences in the material that they might on first thought suggest, but rather to its somewhat capricious nature, resulting in fluctuations of a minor sort which an imperfectly developed technic fails to set forth in true perspective. Success depends in large degree upon the elimination of these more superficial variables by the choice of material in which they are at a minimum.

While the small amount of evidence on some phases of the problem might not recommend such a treatment, the extent of our knowledge of the requirements for pollen-tube growth and the directions in which further research might profitably be made will best be indicated perhaps by classifying the subject matter to permit of separate consideration of the carbon, oxygen, nitrogen, and mineral requirements, water, osmotic, and acidity relations, and the amount and kind of growth obtained.

#### MATERIALS AND METHODS

During the course of these studies the extensive resources of plant material maintained in the Arnold Arboretum, the Harvard Botanic Garden, and at the Bussey Institution have been freely drawn upon. Of the many species tested, but a few were found which lent themselves readily to our purposes. Whenever possible the plants yielding suitable pollen were grown in the greenhouses at the Bussey Institution, and in so far as it could be readily done the supply of pollen was prolonged beyond the usual season. The pollen of *Vinca*, *Scilla*, *Puschkinia*, *Chionodoxa*, *Muscari*, and *Lythrum* used was largely gathered out of doors. Our inability to extend the flowering period of some very desirable forms proved to be a real handicap and is responsible to a certain extent for the incompleteness of some of the experiments reported.

Semi-solid media with agar as the basis were found the most suitable in cultivating pollen artificially. Except where otherwise mentioned, common shred agar and granulated cane sugar of the trade were employed. A petri dish in which a thin layer of agar is poured and allowed to set may be readily handled on the stage of a binocular microscope while the pollen is being plated. With the aid of a needle, a heap of grains transferred to the culture can be separated with facility and arranged in regular lines.

When thus oriented, subsequent examinations may be made systematically without danger of confusion. Such an arrangement is especially convenient when it is necessary to make repeated measurements on each of a series of tubes, as in securing data for growth curves.

Except in a few of the early experiments in which a lower power was used, the pollen tubes were measured with a 7.5 ocular micrometer used in conjunction with a 16-mm. objective. Where the pollen tubes growing on artificial media take a rather straight course or do not change their direction frequently, their lengths may be measured to within  $40\ \mu$ . Where there is considerable deviation from a straight line, the error is, of course, larger; but among such forms as *Cucumis*, *Scilla*, and *Vinca*, such tubes are not very frequent. With practice even these can be measured with a fair degree of accuracy. Whenever possible, average values have been obtained based on the measurement of from 50 to 125 tubes. When the tests are repeated also, this procedure overcomes to a considerable extent, we believe, the inherent shortcomings of the material for such quantitative experiments as we have made. In obtaining points for growth curves it was found necessary to make frequent, and, if a number of tubes were involved, rather rapid, measurements during the early stages of elongation. Very fortunately, pollen tubes of *Scilla*, *Vinca*, and *Cucumis* during this period maintain a fairly straight course, and their lengths may be readily determined to within  $25\ \mu$ . As a tube grows longer its direction may be rather frequently altered, but in the forms mentioned, in marked contrast to the vagrant tubes of *Hippeastrum*, for example, these digressions do not present serious difficulties.

The cultures were usually grown at room temperature and were set away in a drawer or covered on the table. These latter precautions were perhaps unnecessary, since the diffused light of the laboratory was apparently without influence on growth.

#### CARBON REQUIREMENTS

It is a fundamental and familiar fact that, in the formation of the organic material of the green plant,  $\text{CO}_2$  serves as the primary source of carbon. It is the chloroplasts alone, and these only when they contain chlorophyll, that are active in its assimilation. Plants devoid of chlorophyll, or particular organs lacking it, are wholly unable to utilize the carbon supply of the atmosphere. That the formation, however, of the widely distributed reserve food material, starch, does not necessarily stand in direct connection with this assimilation of  $\text{CO}_2$  is shown by the fact that green leaves floated upon sugar solution of suitable concentrations in the dark will form starch. Even in the green plant, then, sugar may be interposed as a source of carbon. It is commonly accepted that in plants lacking chlorophyll, such as the fungi, sugars obtained *ab extra* regularly serve to fill the carbon requirements. Within the body of the green plant, the cells remote from the

chlorophyll tissues derive their supplies of carbon from the sugars moved from the green parts. No chlorophyll is present in the pollen tube, of course, and beyond the reserves in the pollen grain it likewise is dependent upon outer sources for its carbon supply. There is good presumptive evidence that it derives these directly from the stylar tissues in the form of readily diffusible sugars. The results of studies on pollen-tube nutrition *in vitro* throw considerable light on this phase of the problem. Since Schleiden (1849) and Van Tieghem (1869) made the first serious attempts to cultivate pollen artificially, cane-sugar solutions have been frequently employed as media and with some success. Of the numerous studies that have been reported, however, a few only contribute evidence of critical value in determining the part played by sugar as a nutrient. The reserve materials of the pollen grains themselves are probably sufficient to support an appreciable amount of growth, and it must be considered that the sugars added might by a purely osmotic action so adjust the physical conditions of the medium that growth could proceed until this stored material was exhausted. In pollen cultures where the amount of growth obtained is meagre—and these unfortunately comprise most of the reported cases—it is impossible, therefore, to define with certainty the action of a substance incorporated in them. If, however, on the addition of material of nutritive value, growth results clearly in excess of the amount that the reserve materials of the pollen grains could possibly support under the most favorable physical conditions, it would be justifiable to conclude that the added substance had actually been assimilated and had contributed to the formation of the pollen tube. A few examples of this sort are to be noted.

Jost (1907) secured pollen tubes of *Hippeastrum aulicum* 17–22 mm. in length on agar cultures containing 1 percent of cane sugar. When 1/4–1/2 percent of glucose was substituted for the cane sugar, the tubes attained a length of 7–8 mm. Knight (1917) reports that Miss Eckerson secured some pollen tubes of apple 10 mm. in length in 3-percent solutions of fructose. Bobilioff-Preisser (1917) found that the pollen of *Vinca minor* in 1–10 percent cane-sugar solutions formed tubes 10 mm. in length. The writer, working with pollen from an *Hippeastrum* plant of hybrid origin, has secured numerous tubes over 10 mm. in length on 2-percent agar cultures containing 3 percent of cane sugar. Very commonly two tubes are emitted from a single pollen grain in this form the total length of which frequently exceeds 10 mm. Pollen of *Vinca minor* cultured on 2-percent agar with 5 percent of sugar added produced tubes 7 mm. long. In *Nicotiana glauca* some tubes reached a length of over 100 times the diameter of the pollen grain on agar plates containing .15 percent cane sugar, and in *Scilla*, in which the pollen grain measures about 50  $\mu$  through its longest axis, tubes were secured, on agar cultures to which only cane sugar had been added, 4 mm. in length or about 80 times as long as the pollen grain itself.

While we have no exact quantitative evidence in these cases to show



that the amount of dry matter has actually been increased during growth, microscopic observation strongly favors such a conclusion. Tubes are formed with cellulose or callose walls reaching a length in some cases equal to several hundred diameters of the pollen grain and containing in their frequently bulbous tips a considerably enhanced mass of protoplasm. These tubes, moreover, commonly form numerous callose plugs the total volume of which may alone exceed that of the grain. The inference is clear that the sugars in the medium have been drawn upon in the development of these structures. It would appear also that sucrose, glucose, or fructose may serve as sources of carbon although all are not equally suitable in a given case.

There is another line of evidence which also points to the conclusion that sugars are taken up by pollen tubes. Mangin (1886) first noted that pollen grains of some species when placed on sugar cultures deposited starch freely. Tischler (1917) has confirmed this observation. Dodel-Port (1880) also found that pollen of *Pinus Laricio* on concentrated sugar solutions formed numerous large amylum grains. Green (1894) observed that the fresh pollen of *Zamia* contains no starch but that it appeared regularly in the grains when cultured with sugar. While these facts show that pollen grains may take up sugar and deposit starch initially, and suggest that the process of sugar-absorption may be maintained throughout the growth of the tube, we should not expect, for reasons to be considered later, that the deposition of starch would continue during growth under artificial conditions. Such a deposition is not, of course, a necessary consequence of sugar-absorption, and failure to detect starch in growing tubes would not vitiate the conclusion that sugars from without were being utilized.

#### OXYGEN REQUIREMENTS

Van Tieghem (1869) first demonstrated the necessity of oxygen for germination. He sowed pollen in a drop of water, placed a cover glass over it, and noted that only the grains at the periphery emitted tubes. When the cover glass was lifted, the remainder grew. Van Tieghem also secured quantitative evidence showing that during the elongation of the pollen tube, accompanied by the digestion of the starch within it, oxygen was removed from the surrounding air and replaced by carbon dioxide. Mangin (1886) reported that on artificial cultures the respiratory ratio falls gradually from the time of germination, and that, moreover, the quantities of gas taken up or given off decrease gradually until the cell dies. Pollen lacking starch did not readily germinate in media deprived of sugar, and it was found that the respiratory ratio under such conditions was four to five times less than in sugar solutions. While Mangin does not state the nature of the reserve material in these cases, it is probable that in some of them, at least, unsaturated fats were present, the digestion of which would give the low respiratory ratios observed. The marked acceleration of the respiratory processes in the sugar media suggests a direct utilization of this carbohydrate.



## THE EFFECT ON TOMATO, SOY BEAN, AND OTHER PLANTS OF ALTERING THE DAILY PERIOD OF LIGHT

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The results of experiments along this line with a number of different species of plants were published in the *Annals of Botany*, volume 37, January, 1923. Some further experiments were continued during the year 1922 with tomato, soy bean, hemp nettle (*Galeopsis Tetrahit* L.), and shamrock (*Trifolium dubium* Sibth.). In all the experiments care was taken to have all the conditions, except the amount of light, as uniform as possible.

### TOMATO

There were 10 pots, each of which contained a single plant. Two varieties were used for experiment. Two pots of one variety and three pots of the other variety were exposed to light, and an exactly similar set was darkened periodically. The pots were darkened on various dates between the 3d and the 20th of May for a total period of  $53\frac{1}{2}$  hours. The time of darkening on any one day varied from one to six hours. The average length of day during this period, measured from sunrise to sunset, was 14 hours, 52 minutes. The effect of darkening the plants was to reduce the length of daylight to which they were exposed to a daily average of 11 hours, 54 minutes.

The heights of the plants at the beginning and end of the experiment and the dates of flowering were as follows in the two varieties:

*First Variety:* The average height of the two plants exposed to light was 42.5 mm. on May 3, and 324.0 mm. on June 1, and they came into flower on May 30 and June 1 respectively.

The average height of the plants darkened periodically was 42.0 mm. on May 3 and 273.5 mm. on June 1, and they came into flower on May 30 and June 1 respectively.

*Second Variety:* The average height of the three plants exposed to light was 31.0 mm. on May 3 and 250.0 mm. on June 12, and they came into flower on June 8 (one plant) and June 10 (two plants).

In the case of the plants darkened, the average height was 30.3 mm. on May 3 and 256.6 mm. on June 12, and they came into flower on June 8 (two plants) and June 10 (one plant).

### SOY BEAN

Three varieties were used for this series of experiments. The seeds were sown in rows in the open ground, there being two rows of each variety,

one row to be darkened periodically and the other to be exposed to the full period of daylight. The seeds were sown on May 10, and the period of darkening extended from May 29, when the plants were 2 inches high, until June 14. The alternate rows were covered altogether for 60 hours, the time of covering on any one day varying from 2 to 7 hours. The length of the day, measured from sunrise to sunset, during this period was  $15\frac{1}{2}$  hours, while the result of darkening the plants was to reduce the daily period of light to 12 hours. In two varieties there were thirteen plants in each row, while in the third variety there were only 8 plants in each row during the period of the experiment.

The time of coming into flower and the number of plants in flower on a particular date were as follows:

*First Variety:* In the row exposed to light the first flower opened on July 11, while on July 13 all the 13 plants were in flower.

In the darkened row the first flower opened on July 7, while on July 13 11 plants were in flower.

*Second Variety:* In the row exposed to light the first flower opened on July 11, while on July 13 all the 13 plants were in flower.

In the darkened row the first flower opened on July 7, while on July 13 all the 13 plants were in flower.

*Third Variety:* In the row exposed to light the first flower opened on July 8, while on July 18 all the 8 plants were in flower.

In the darkened row the first flower opened on July 8, while on July 18 7 plants were in flower.

#### HEMP NETTLE

There were 12 pots, 6 of which contained one seedling each of a purple-flowered variety and 6 contained one seedling each of a white-flowered variety. The pots were darkened on various dates between the 2d and the 31st of May for a total period of  $83\frac{1}{2}$  hours, the time of darkening on any one day varying from one to seven hours. The average length of day during this period, measured from sunrise to sunset, was 14 hours, 50 minutes. The effect of darkening the plants was to reduce the length of daylight to which they were exposed to a daily average of 12 hours, 3 minutes.

Observations on these plants were made from May 2 to July 8. The heights and number of leaves were noted at the beginning of the experiment, also the time of flowering, and the height was noted at the end of the experiment. For purposes of comparison two half-grown leaves were taken as equal to one full-sized leaf.

*Purple-flowered Plants:* The average height of the 3 plants exposed to light was 123.3 mm. on May 2, the average number of leaves per plant on May 2 was 7, and the average height on July 8 was 670.3 mm. The first flowers on each plant opened on July 5.

The average height of the 3 darkened plants was 126.6 mm. on May 2, the average number of leaves per plant on May 2 was 7, and the average height on July 8 was 697.3 mm. The first flowers opened on July 5 (two plants) and July 6 (one plant).

*White-flowered Plants:* The average height of the 3 plants exposed to light was 98.3 mm. on May 2, the average number of leaves per plant on May 2 was 7, and the average height on July 8 was 670.0 mm. The first flowers opened on July 3 (two plants) and July 8 (one plant).

The average height of the 3 darkened plants was 98.3 mm. on May 2, the average number of leaves per plant on May 2 was 7.3, and the average height on July 8 was 762.6 mm. The first flowers opened on July 6 (two plants) and July 7 (one plant).

#### SHAMROCK

There were ten pots each containing one plant, all the plants being of approximately equal size. The period of darkening extended from May 15 to June 12, the time of darkening on any one day varying from one to seven hours. The total number of hours during which the plants were covered was 95. The length of daylight during this period averaged 15 hours, 6 minutes. The effect of darkening was to reduce the daily period of light to 11 hours, 43 minutes.

The 5 plants exposed to light came into flower on various dates from the 13th to the 29th of June, while those darkened came into flower on different dates from the 23rd of June to the 17th of July. The average date of coming into flower in the case of the darkened plants was 11 days behind that of the plants exposed to light.

#### SUMMARY

Experiments were conducted at Ottawa, Canada, on the result of shortening the average period of daylight from about 15 hours (the natural length of day) to about 12 hours. The period of darkening the plants extended from May 2, 1922, to June 12, 1922, altogether, but was not the same in each of the four species experimented with.

In the case of tomato, both sets of plants came into flower about the same time.

In the soy bean the plants darkened came into flower a little earlier.

The hemp nettle plants exposed to light flowered a little earlier than the others, but the darkened set were somewhat taller.

In the case of the shamrock plants, the effect of darkening was to delay the average date of flowering for a period of about 11 days.

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CENTRAL EXPERIMENTAL FARM, OTTAWA

# A REVISION OF THE GENUS NEMACLADUS (CAMPANULACEAE)

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## INTRODUCTION

Dissatisfaction with the inability to identify, with any certainty, material collected of the genus *Nemacladus*, and with the identification of the material in the herbaria visited, led me to undertake the revision presented in this paper. The small size of the flowers, the variability of the taxonomic characters, and the rather complete concealment of the corolla within the calyx in dried specimens, all make such study rather difficult. Even after several months of work on the group, I am able to make certain determination only after boiling and dissecting the flowers.

For this study, material has been available from many sources. In citing specimens in different herbaria, the following abbreviations indicated in parentheses are used: C. F. Baker Herbarium of Pomona College (Baker), California Academy of Sciences (Cal. Acad.), Dudley Herbarium of Stanford University (Dudley), Gray Herbarium (Gray), Missouri Botanical Garden (Mo.), New York Botanical Garden (N. Y.), Herbarium of F. W. Peirson of Pasadena, Calif. (Peirson), Philadelphia Academy of Natural Sciences (Phila.), Rocky Mountain Herbarium (Wyo.), United States National Museum (U. S.), University of California (U. C.), and Washington State College (Wash.). To those in charge of the herbaria listed above, I take this opportunity of expressing appreciation of the kindness shown in lending material, and to Mr. Ivan M. Johnston of Gray Herbarium for looking up certain references.

## DISCUSSION OF THE GENUS

The original description of the genus by Nuttall was for the species *ramosissimus* and has had to be modified at various times as new forms have been discovered. Nuttall considered it as having no close affinity and proposed the "order" Nemacladaceae, "probably between the true Lobeliaceae and Goodeniaceae proper." Torrey placed it in the Lobeliaceae, and Bentham and Hooker in the Campanulaceae, in either of which groups it has since remained according to the author's conception of these families. Gray in 1875 indicated the relationship to the South African *Cyphia*.

In this paper I have accepted the narrow generic limit made by Greene (*Erythea* 1: 237-238. 1893) when he creates for *N. oppositifolius* Robinson (Proc. Amer. Acad. 26: 168. 1891) the genus *Baclea*. If *Baclea oppositi-*

*folia* is to be included in *Nemacladus*, so must be *Parishella californica* Gray. These three genera of southwestern North America can be compared as follows:

	Baclea	Nemacladus	Parishella
Duration	Perennial	Annual	Annual
Leaves	Cauline	Mostly basal rosette	Basal and cauline
Seeds	Flattened Smooth	Cylindrical Pitted and ridged	Cylindrical Pitted and ridged
Glands on ovary	Absent	Present	Present
Stamineal appendages	Absent	Present	Present
Dehiscence of capsule	By valves	By valves	Circumscissile

The seeds of *Parishella* are not smooth as described by Gray (Proc. Amer. Acad. 19: 84. 1883), but exactly like those of *Nemacladus rigidus* var. *interior*, and the glands and appendages, although not mentioned in descriptions of *Parishella*, are very well represented. Apparently the first botanist to see these in *Nemacladus* was Parish. It is unfortunate that his species *adenophorus*, which was more carefully described than perhaps any other (Bull. S. Cal. Acad. 2: 28. 1903), has to be reduced to synonymy; his is the only description mentioning the glands and appendages, although the number of glands is three instead of four and the appendages come from the filaments between the glands and not from the glands themselves.

Of the ten species that have been described in *Nemacladus*, *sensu stricta*, two (*tenuissimus* Greene and *adenophorus* Parish) are synonyms. The other eight and two additional forms are here grouped in three species: (1) *N. longiflorus*, with long tubular corolla and almost separate sepals; (2) *N. ramosissimus*, with open and more regular, campanulate corolla, but petals still well grown together, and with stamineal appendages with projecting terminal cells. (3) *N. rigidus*, with petals almost separate and stamineal appendages with pendant terminal cells.

To *ramosissimus* as varieties are referred *N. pinnatifidus* and *N. gracilis*; to *rigidus* as varieties, *N. montanus*, *N. capillaris*, *N. rubescens*, and two new ones: *interior* and *australis*.

In arranging these ten aggregates under three species, I am well aware that many botanists would prefer to make them all separate species. Indeed, my own feeling on first studying the types and making the drawings in Plates IX and X was such. But the types represent the extremes of the several lines of divergence, hence the differences shown in my drawings are not constant enough to warrant specific rank for all ten concepts. The

number of intergrades and of variants in all directions is too great to permit this. Yet I can not conceive of these intermediate forms as being to any extent hybrids; there is for the most part insufficient overlapping of range for this, and the extremely minute flowers are not likely to be cross-pollinated to any extent. In using the name *rigidus* for the third species, I realize well that *rigidus* proper is not as representative of all the groups in this third lot as perhaps *interior* or *rubescens*, but I use the name because it is the first one published for any of the six groups here included.

The center of distribution for the genus seems to be coastal southern California with *N. ramosissimus* and its two varieties and with *N. longiflorus*. To the north and east occur *N. rigidus* and its varieties, the only overlapping of range being in the mountains along the edge of the desert, where *gracilis* and *rubescens* are found together, and in the occurrence of *N. rigidus* var. *australis* in northern Lower California.

#### DESCRIPTION OF THE GENUS

NEMACLADUS. Nuttall, Trans. Amer. Phil. Soc. n. s. 8: 254. 1843. Walp., Ann. 2: 1038. Torrey, Mex. Bound. Survey 108, Pl. 35. 1859. Gray, Jour. Linn. Soc. 14: 28. 1875. Bentham and Hooker, Genera Plantarum II, 2: 554. 1876. Gray, Proc. Amer. Acad. 12: 60. 1876. Gray Synop. Flora II, 1: 2. 1878, and Suppl., p. 393. 1886. Gray, in Brewer & Watson, Bot. Calif. 1: 445. 1880, and 2: 460. 1880. Schönland, in Engler and Prantl, Nat. Pflanzenfam. IV, Abt. 5: 62. 1894. Abrams, Flora Los Angeles, 387. 1904, and ed. 2, p. 354. 1917.

Small annual herbs with fine, generally diffusely branched stems. Basal leaves in a compact rosette, cauline leaves largely reduced to subulate bracts. Flowers borne singly on filiform pedicels scattered in racemose fashion along the branches. Flowers generally erect; calyx partly or wholly free from ovary; corolla varying from having petals scarcely united to quite tubular, more or less distinctly bilabiate, lower lip two-, upper three-lobed. Stamens monadelphous above, curved at the end and with the five anthers stellately spread around the two-lobed stigma. Ovary with three flattened, rounded glands near the base, these opposite the three lobes of the upper lip of the corolla. The two staminal filaments between these glands with small appendages, each stipe-like and with one or more terminal, transparent, rod-like cells. Capsule largely two-celled, dehiscing by valves. Seeds cylindrical, with longitudinal ridges and transverse lines dividing the surface into pits or cells.

Generic type: *N. ramosissimus*. Nutt.

#### KEY TO THE SPECIES

- A. Corolla tube narrow, usually exceeding calyx;  
 calyx not adnate to ovary, well separated into  
 distinct sepals; mature capsule fusiform, 2-3  
 times the length of the calyx..... 1. *N. longiflorus* Gray.



- 4A. Corolla, when petals are united, not narrow, but campanulate; calyx adnate to ovary and with distinct lobes only as long as the tube; mature capsule never much longer than calyx.
- B. Corolla with petals united for  $\frac{1}{2}$  to  $\frac{1}{3}$  its length; staminal appendages with very slender transparent cells, which extend straight out from the base so as to appear fan-like.
- C. Stems perfectly straight, not at all zigzag; basal leaves dentate; corolla about twice the length of the calyx; seeds short, almost spherical, and with only about 6 cells in each longitudinal row. . . . . 2. *N. ramosissimus* Nutt.
- CC. Stems slightly zigzag; corolla generally not much longer than calyx; seeds distinctly longer than round, with 8 or more cells per row.
- D. Basal leaves pinnatifid, with the lobes toothed; capsule acute; sepals long, narrow. . . . . 3. *N. ramosissimus* var. *pinnatifidus* (Greene) Gray.
- DD. Basal leaves dentate to entire; capsule rounded. . . . . 4. *N. ramosissimus* var. *gracilis* (Eastw.) Munz.
- BB. Corolla with petals united only at very base. Staminal appendages with somewhat clavate, rather thick, transparent cells which are at right angles to basal piece.
- C. Plant compact, very robust; calyx and ovary much enlarged in fruit, about  $2\frac{1}{2}$  times as long as when in flower.
- D. Corolla scarcely exceeding calyx; basal leaves entire, fleshy. Range from eastern Oregon to west central Nevada. 5. *N. rigidus* Curran.
- DD. Corolla much longer than calyx; basal leaves serrate, apparently not fleshy. Northern Lower California. . . . . 6. *N. rigidus* var. *australis* Munz.
- CC. Plant diffuse, or, if small, not robust; calyx and capsule not much longer in fruit than in the flower.
- D. The three petals of the upper lip generally ciliate; stamens well exerted; pedicels not conspicuously ascending, rather spreading and sinuous. Deserts from California into Nevada, Utah, and New Mexico. . . . . 10. *N. rigidus* var. *rubescens* (Greene) Munz.
- DD. The petals not ciliate; stamens about as long as petals; pedicels usually straight and ascending. California, chiefly from regions north of the deserts.

- E.* Calyx tube in fruit long-turbinate below; seeds few, 5-12; capsule swollen above, exceeding calyx; flowers small, about 2 mm. long; stem usually not strongly zigzag... 9. *N. rigidus* var. *capillaris* (Greene) Munz.

*EE.* Calyx tube in fruit rounded below, seeds more numerous; flowers about 3 mm. long; stem strongly zigzag.

- F.* Seeds large, about 900 microns long, with broad, flattened ridges, each of these with about 30 cells. Butte County to Napa County, California..... 7. *N. rigidus* var. *montanus* (Greene) Munz.

*FF.* Seeds smaller, 550-650 microns long and with low, narrow longitudinal ridges, between which lie rows of about 10 large, fairly regular cells. Lower western slopes of the Sierras south to Kern County, California..... 8. *N. rigidus* var. *interior* Munz.

1. *N. LONGIFLORUS* Gray, Proc. Amer. Acad. 12: 60. 1876. Gray, Synop. Flora II, 1: 3. 1878, and Suppl., 394. 1886. Gray, in Brewer & Watson, Bot. Calif. 2: 460. 1880. Hall, Univ. Calif. Pub. Bot. 1: 123. 1902.

(Plate IX, figs. 1-7)

Simple to diffusely branched from near base; stems 3 to 20 cm. long, glabrate, sometimes slightly zigzag. Basal leaves narrowly spatulate to obovate, 3 to 15 mm. long, mostly entire, sometimes denticulate, densely white-pubescent; cauline leaves subulate to narrowly lanceolate and 1-5 mm. long. Inflorescence with spreading to recurved pedicels of 10 to 25 mm. length. Flowers 4-7 mm. long; calyx divided almost to base, free from ovary, generally 1-3 mm. long, sepals lanceolate and generally glabrous. Corolla white, 2-4 times the length of the calyx, tubular half way, limb distinctly labiate, 2 lower lobes shorter than 3 upper, upper lip bearded at entrance to throat and with yellowish spot at base of each lobe. Corolla lobes in dried specimens richly veined and with many closed cells; often tinged pink. Stamens barely exerted, pubescent near anthers; staminal appendages near upper end of ovary, very small and with 2-3 fine transparent cells; anthers slightly bluish. Ovary fusiform, the three glands elongate. Fruiting capsule 2-3 times the length of the calyx and 2-4 mm. long. Seeds small, 300-350 microns long, slightly longer than thick, with 9 or 10 weak ridges and about 10 cells between each two consecutive ridges.

*Distribution:* coastal slopes of southern California from Los Angeles County into Lower California, ascending to altitudes of 6000 ft. (Mrs. Spencer 1661, Baker). Types: from "S. E. California, Wallace, Lemmon."

*Specimens examined:*

California: California, *Wallace* (Gray); California, *Lemmon in 1876* (Gray, N. Y.); southern California, *Parry & Lemmon in 1876* (Gray, Mo., N. Y., Phila.); Los Angeles Co., *L. T. Chamberlain* (N. Y.); Roscoe, *Eastwood 232* (Cal. Acad., Gray, Mo.) and *269* (Cal. Acad.); San Fernando Wash, *Eastwood 3159a* (Cal. Acad.); Arcadia, *Grant in 1902* (Dudley, Gray, U. C.); Rubio Wash, San Gabriel Mts., *Peirson 220* (Peirson); west fork of San Gabriel River, *Peirson 2453* (Peirson); San Bernardino, *Parish in 1893* (Mo.), *in 1896* (Mo.), *in 1897* (N. Y.), *in 1905* (U. C.), *1339* (Mo.), *3441* (U. S.), *3651* (Gray, U. C.), *5378* (Phila.), *5391* (Gray, Wyo.), *7081* (Baker, U. C.), *11364* (Baker); San Bernardino (?) *Vasey in 1880* (U. S.); San Bernardino, *Lemmon in 1876* (U. C., U. S.); San Bernardino, *R. J. Smith in 1903* (Phila., U. C.); Mt. San Bernardino, *R. J. Smith in 1903* and *1904* (U. C.); Crafton, *Lemmon* (Dudley, Gray, Mo., U. S.); San Bernardino Co., *Lemmon & wife in 1876* (Mo.); Highland, *Mrs. Spencer* (Baker); Colton, *M. E. Jones 3185* (Baker, Cal. Acad., Mo., U. S., Wyo.) and *3395* in part (Baker, Wyo.); Colton, *Parry in 1881* (Gray, U. C.); and *369* (Mo.); Colton, *Pringle in 1882* (Mo., Phila., U. S.); Colton, *Nevin 539* (Gray); Mohave desert, *S. B. & W. F. Parish 1339* (Gray, U. S.); Covington ranch, Little San Bernardino Mts., *Munz & Johnston 5165* (Baker); near Riverside, *K. Brandegee in 1905* (Baker, U. C.); Banning, *M. E. Jones in 1903* (Baker); San Jacinto Mts., *S. B. & W. F. Parish 1628* (Gray); Strawberry Valley, *Hall 960* (U. C.) and *F. M. Reed 2409* (Wyo.); Chalk Hill, *Hall 2047* (Dudley, U. C.); Onstatts Valley, *Hall 661* (U. C.); Tahquitz Trail, *Mrs. Spencer 1661* (Baker) and *Jaeger 1178* (Baker); Temescal Canyon, *Munz 5024* (Baker) and *Peirson 2920* (Peirson); Elsinore, *McClatchie in 1892* (N. Y.); Palomar, *Peirson 2184* (Peirson) and *Hall 1960* (Dudley, Phila., U. C., U. S.); Potrero, *Orcutt 1551* (Mo.); Vulcan Mt. near San Felipe, *Hall 1199* (Mo., U. C.); Banner, *M. E. Jones in 1906* (Baker); Witch Creek, *Alderson in 1894* (Baker, U. C.); Masons, La Puente Valley, *T. S. Brandegee in 1895* (U. C.); Buckmans Springs, *Ethel Campbell 27* (Cal. Acad.); Colorado desert, *Alvina Buttle in 1912* (Cal. Acad.); Campo, *Abrams 3738* (Dudley, Gray, Mo., Phila., U. S.) and *Cleveland 439* (U. C.); between Jacumba and Mt. Springs, *Eastwood 9513* (Cal. Acad.); Valle de las Viejas, *Cleveland in 1877* (Gray); San Diego, *Babcock* (Dudley).

Lower California: Lower California, *Orcutt in 1883* (Mo.) and *in 1884* (U. S.); Nachoguero Valley, *Schoenfeldt 3420* (Dudley, U. S.); Hanson's ranch, *Orcutt in 1884* (Gray, U. C., U. S.).

This species varies considerably in length of calyx, corolla, and capsule. In plants from the more outlying parts of its range, all these parts are apt to be short. In the Brandegee specimen from Masons and in *Eastwood 9513* the sepals are abnormally long and in the Eastwood specimen very pubescent; all other plants studied had a practically glabrous calyx. The reference by Hall gives a very excellent field description.

2. *N. RAMOSISSIMUS* Nutt. in Trans Amer. Phil. Soc. n. s. 8: 254. 1843.  
Gray, Synop. Flora II, 1: 3. 1878, in part.
- N. tenuissimus* Greene, Bull. Cal. Acad. 1: 198. 1885.
- N. ramosissimus montanus* (Greene) Gray in Abrams, Flora Los Angeles, 388. 1904, and ed. 2, p. 354. 1917, in part.

(Plate IX, figs. 8-16)

Simple, to diffusely branched in larger specimens; stem up to 25 cm. long, not at all zigzag; glabrous or puberulent. Basal leaves 1-3 cm. long, linear to lanceolate-ovate, strongly dentate, glabrous or pubescent. Lower cauline leaves sometimes toothed, upper ones generally less than 1 cm. long and subulate. Inflorescence with spreading or recurved, capillary pedicels about 1 cm. long. Calyx 1.5 mm. long, adnate to ovary, its segments lanceolate and of length equal to that of tube. Flowers 2.5 mm. long, with corolla tubular for about two fifths its length; corolla lobes quite equal and about twice the length of the calyx, white or tinged with dark red. Stamens scarcely equal to the corolla, generally hairy at the curve; staminal appendages small, with several very slender clear cells. Ovary broad, about 3 mm. long in fruit and slightly exceeding the calyx. Seeds about 500 microns long, short, almost round, with 10 rows of about 6 cells each.

*Distribution:* California, chiefly coastal parts from Los Angeles south; two collections known from Monterey County. Sandy soil and dry slopes below 5000 feet altitude. Type: *Nuttall*, "near San Diego."

*Specimens examined:*

California: California, *Orcutt in 1886* (U. S.) and *Lemmon 1241* (Gray, Mo.); Tassajara Hot Springs, Monterey Co., *Elmer 3350* (Dudley, Mo., U. S.); Tassajara, *Dudley in 1901* (Dudley); Rubio Canyon, San Gabriel Mts., *Peirson 220* (Peirson); San Dimas Canyon, *Munz & Harwood 3695* (Baker), *3797* (Baker), *3827* (Baker, Dudley, Wyo.); Mohave desert, *Lemmon & wife in 1884*, in part (U. S.); between San Bernardino Mts. and Mohave River, *Parry & Lemmon 218* (Mo., N. Y.); San Bernardino, *S. B. & W. F. Parish 1034* (Gray), *831* in part (U. C., U. S.); *S. B. Parish in 1885* (Mo.), *in 1888* (U. C.), *3829* (Mo., U. C.), *5621* (U. C., Wyo.); San Bernardino Mts., *S. B. & W. F. Parish 939* (Gray, U. S.); San Bernardino valley, *S. B. & W. F. Parish 1338A* (Gray); Arrowhead, *Grant 6639* (Dudley), *Colton, Parry 161* (Gray); Colton, *M. E. Jones 3395*, in part (Baker, Mo., U. S.); Bloomington, *Parish 11266* (Baker, Gray); Riverside, *Mrs. Wilder 575* (Baker); Kenworthy, San Jacinto Mts., *Munz & Johnston 5503* (Baker); Hemet Dam, *Mrs. Wilder 967* (U. C.); Murietta, *Munz & Johnston 5320* and *5360* (Baker); Palomar, *Hall 1961a* (U. C.); Witch Creek, *Alderson in 1894* (U. C.); Ramona, *T. S. Brandegee in 1894* (U. C.); Campo, *Abrams 3592*, in part (Dudley, Mo.); Jamul, *Orcutt in 1885* (U. C.); Colorado desert, *T. S. Brandegee in 1901* (U. C.); San Diego, *Nuttall* (Gray, Phila.); San Diego, *Pringle in 1882* (Phila., U. S.).

Lower California: All Saints Bay, *Greene in 1885* (U. C.); La Grulla, *Orcutt in 1886* (Mo., U. S.); Guadalupe Mts., *Orcutt in 1883* (Gray).

The most characteristic features of this species in its typical form are the strict absence of any zigzag condition in the stem, and the shortness of the seeds. Many of the references in the literature to this species do not apply to it at all; for example, Torrey's plate in the Mexican Boundary Survey (Pl. 15. 1859) shows a specimen of *N. rigidus* var. *rubescens* under the name *ramosissimus*.

3. *N. RAMOSISSIMUS* var. *PINNATIFIDUS* (Greene) Gray, Synop. Flora, Suppl. II, 1: 393. 1886. Abrams, Flora Los Angeles, 388. 1904, and ed. 2, p. 355. 1917.

*N. pinnatifidus* Greene, Bull. Calif. Acad. 1: 197. 1885.

(Plate IX, figs. 17-23)

Habit of the species, but with the stem slightly zigzag and with basal leaves pinnatifid, the lobes being toothed. Flowers much as in the species, corolla lobes slightly exceeding the sepals. Mature capsule rather acute, 3-6 mm. long. Seeds about 600 microns long, with 10 rows of 8-10 cells per row.

*Distribution*: southern California, mostly on the coastal slopes from Los Angeles County into Lower California. Type: *Greene*, All Saints Bay, Lower California.

*Specimens examined*:

California: Los Angeles Co., Santa Anita Canyon, Sierra Madre Mts., *Allen in 1885* (Gray); San Gabriel River, *Leiberg 3385* (U. S.); Echo Mt., *McClatchie*, probably this (N. Y.); Rubio Canyon, *Peirson 219* (Peirson); Palm Canyon in Riverside Co., *Peirson in 1920* (Peirson); Palomar, *Hall 1961b* (U. C.); Potrero, *Abrams 3746* (Dudley); San Felipe Creek, *M. E. Jones in 1906* (Baker); Cholla Ranch, *M. E. Jones in 1906* (Baker); San Felipe Creek, *Eastwood 2702* (Cal. Acad., Gray, Mo., N. Y., U. S.); Coyote Wells, *McGregor 845* (Dudley); and s. w. part of the Colorado desert, *Orcutt in 1889*, in part (Gray, U. S.).

Lower California: All Saints Bay, *E. L. Greene in 1895* (Cal. Acad., Gray, U. C.).

The most distinctive features are evidently the pinnatifid basal leaves and the long sepals. The Hall specimens from Palomar are quite intermediate between typical *ramosissimus* and the variety; the Jones plants from San Felipe have the leaves of the species, but the flowers of the variety.

4. *N. ramosissimus* var. *gracilis* (Eastwood) Munz comb. nov.

*N. gracilis* Eastwood, Bull. Torrey Bot. Club 60: 500. 1903.

*N. ramosissimus montanus* (Greene) Gray, Synop. Flora, Suppl. II, 1: 393. 1886 for Southern California material and in part, in Abrams,

Flora Los Angeles, 388. 1904, and ed. 2, p. 355. 1917, and in Hall, Univ. Calif. Pub. Bot. 1: 123. 1902.

(Plate IX, figs. 24-29)

Habit that of the species, but with zigzag stems. Basal leaves dentate to entire, spatulate to ovate, short and pubescent. Pedicels usually recurved and spreading, capillary. Calyx generally longer than in the species, almost equal to the corolla which is like that of the species but occasionally hairy. Corolla white, slightly tinged with pink in the bud. Stamens and ovary as in the species; seeds 500-600 microns long, with 10 low ridges and 8-15 cells per row.

*Distribution:* California from Monterey County and western Fresno County southward and eastward along the edge of the southern California deserts into Nevada and Arizona. Dry slopes and ridges near to and in the mountains bordering the deserts. Type: *Eastwood* in 1893, Alcalde, Fresno Co. (Cal. Acad.).

*Specimens examined:*

California: Monterey Co., Nacimiento River, *Brewer* 549 (U. C., U. S.); Fresno Co., Alcalde, *Eastwood* in 1893 (Cal. Acad.); Tehachapi Mts., *Davy* 1864 (U. C.); Sunset, *Heller* 7729 (Dudley, Gray, Mo., Phila., U. C., U. S.); Kernville, *T. S. Brandegee* (Gray); Caliente, *T. S. Brandegee* in 1891 (Baker, U. C.); Panamint Mts., *Coville & Funston* 811 (U. S.); Chat, *M. E. Jones* in 1907 (Baker); Lone Pine Creek, *Hall & Chandler* 7212 (U. C.); Bishop, *Heller* 8278 (Cal. Acad., Dudley, Gray, Mo., Phila., U. C., U. S.); Shepherds Canyon, *M. E. Jones* in 1897 (Baker); Darwin, *Jones* in 1897 (Baker); Mt. Pinos, *Hall* 6415 (U. C.); Acton, *Elmer* 4169 (Dudley); Lancaster, *K. Brandegee* (Baker); Little Rock Creek, San Gabriel Mts., *Peirson* in 1921 (Peirson); Mt. Wilson, *Abrams* 1899 (Dudley); San Antonio Canyon, *J. Durkee* (Mo.); Victorville, *M. E. Jones* in 1903 (Baker, U. S.); Cajon Pass, *M. E. Jones* in 1903 (Baker); Victorville, *Johnston* in 1920 (Baker); Hesperia, *Johnston* in 1920 (Baker); Arrowhead Lake, *Mrs. Wilder* 743 (Baker); east base of San Bernardino Mts., *Parish* 3202 (U. S.); Little San Bernardino Mts., *Munz & Johnston* 5191 (Baker); Colo. Desert, *Wright* 1766 (U. C.); Chalk Hill, San Jacinto Mts., *Hall* 2046 (Dudley, Mo. Phila., U. C., U. S.); Santa Ana Mts., *Munz & Keck* 7096 (Baker); San Felipe, *K. Brandegee* in 1899 (U. C.); Campo, *Eastwood* 9458a (Cal. Acad.); Campo, *Abrams*, 3592, in part (Dudley, Gray).

Nevada: Franktown, *M. E. Jones* in 1882 (Baker, U. S.); Eagle Valley, Ormsby Co., *Baker* 1022 (Baker); Reno, *Stokes* in 1903 (U. S.).

Arizona: Central Arizona, *Palmer* 300 (Phila.).

The distinguishing characters are the corolla of *ramosissimus* itself, but a longer calyx, the presence of the zigzag condition in the stems, and non-pinnatifid basal leaves.



The only published name applicable to this concept seems to be *gracilis* Eastwood, but there are so many plants intermediate between it and *ramosissimus* proper, that varietal rank is all that it can be given. *Abrams* 1899, *Mrs. Wilder* 743, and *Munz & Johnston* 5191 all have the shorter calyx of the species, but the stems are zigzag. Some specimens, such as *M. E. Jones, Victor*, in 1903; *M. E. Jones, San Felipe Hill*, in 1906, and *Heller* 8278 suggest Greene's *rubescens* in having hairy corollas, but other characters definitely place them in *gracilis*. Much of the material here included has gone under the name of *N. ramosissimus* var. *montanus*, but the only point of resemblance is the zigzag condition of the stems.

5. *NEMACLADUS RIGIDUS* Curran in Bull. Cal. Acad. 1: 154. 1885. Gray, Synop. Flora, Suppl. II, 1: 394. 1886.

(Plate X, figs. 30-36)

Compact, branching, coarse-stemmed, glabrous or finely pubescent, stems strongly zigzag, 5-15 cm. high; radical leaves spatulate-lanceolate, cauline leaves ovate to subulate. Pedicels coarse, strongly ascending, 10-12 mm. long. Flowers 2-3 mm. long, calyx lobes longer than the adnate tube, calyx very much inflated in fruit and 4 to 5 mm. long. Corolla deeply divided, quite bilabiate, scarcely exceeding calyx, purplish in color. Stamens equaling corolla; staminal appendages with 2 to 4 fairly thick, pendent transparent cells. Ovary in fruit about 4 mm. long; seeds large, 750 microns long, with 10 flattened, longitudinal ridges, each of which has a row of about 15 cells.

*Distribution*: eastern Oregon to central western Nevada. Type: Geiger Grade near Steamboat, Nevada, *K. Curran* in 1884.

*Specimens examined*:

Oregon: Malheur River, *Cusick* 1625 (Gray, Mo., U. C., U. S., Wash.); Harper Ranch, Malheur Co., *Leiberg* 2113 (Cal. Acad., Gray, Mo., N. Y., U. C., U. S.)

Nevada: Between Reno and Verdi, *Sonne* in 1890 (U. C.); Reno, *Sonne* in 1890 (Mo., U. C.); Reno, *F. H. Hillman* (Baker); Reno, *K. Curran* in 1884 (Dudley, Gray, Phila.); Verdi, *Heller* 10893 (Dudley, Gray, Mo., Phila., U. S.); Steamboat, *K. Curran* in 1884 (Cal. Acad., U. C.).

The robust form and much-enlarged calyx and capsule, in fruit, distinguish the typical form of the species from all others except the var. *australis*, from which it differs in the small corolla and the fleshy, entire leaves. The plant is said to have a general purplish cast.

6. *N. rigidus* var. *australis* var. nov.

(Plate X, figs. 37-40)

Habit and size that of the species. Radical leaves spatulate-lanceolate, coarsely serrate; cauline leaves are narrow bracts. Flowers 3 to 4 mm. long, corolla well exceeding calyx; 3 upper lobes ciliate. Calyx and corolla much inflated in fruit, 4-5 mm. long; stamens shorter than corolla. Seeds large, 750 microns long, with 10 flattened ridges, each with about 30 fine transverse lines.



*Distribution:* northern Lower California. Type: Rosario, Lower Calif., Orcutt 1348 (Gray Herbarium).

*Specimens examined:*

Lower California: Rosario, Orcutt 1348 (Gray, Mo., U. C.).

The robust, compact form, heavy pedicels, much enlarged mature ovary and calyx are quite the same as in the species itself, but the much longer corolla, the ciliate corolla lobes, the very different seeds, which are almost identical with those of *N. rigidus* var. *montanus*, and the isolated range set it apart as a well marked variety.

7. *N. rigidus* var. *montanus* (Greene) Munz comb. nov.

*N. montanus* Greene in Bull. Cal. Acad. 1: 197. 1885.

*N. ramosissimus* var. *montanus* (Greene) Gray, Synop. Flora, Suppl. II, 1: 393. 1886, in part.

(Plate X, figs. 41-46)

Diffusely branched, up to 22 cm. high; stems heavy to slender, glabrous or minutely puberulent, strongly zigzag. Basal leaves oblanceolate, glabrous, dentate; pedicels strongly ascending. Flowers white, about 3 mm. long; corolla deeply divided, its lobes almost twice the length of the calyx lobes. Ovary and calyx approximately equal in fruit, not so enlarged as in the species, scarcely 3 mm. long. Seeds large, about 900 microns long, with broad, flattened ridges, each with about 30 fine transverse lines.

*Distribution:* mountains of middle California from Butte County southwest. Types: Butte County, *Elisha Brooks*, and Lake County, *D. Cleveland*.

*Specimens examined:*

California: Enterprise, Butte Co., *Brooks* (Cal. Acad., U. C.); Allens Springs, Lake Co., *Cleveland in 1882* (Cal. Acad., Gray, U. C.); Napa Co., *Cornelia Masters in 1915* (Cal. Acad.); California, in Jones collection (Baker).

The less compact and less robust habit easily distinguish this from *N. rigidus*; a first glance hardly seems to make it a variety of that species. But the structure of the corolla (the flowers in *N. rigidus* are not enlarged as is the fruit) and the similarity to the seeds of *australis* show a close relationship. The seeds alone distinguish it from var. *interior*, to which is referred the bulk of the material that has been classified under *montanus*.

8. *N. rigidus* var. *interior* var. nov.

*N. ramosissimus* var. *montanus* (Greene) Gray, Synop. Flora, Suppl. II, 1: 393. 1886, for material from the western slopes of the Sierras.

(Plate X, fig. 47)

Habit of *N. rigidus* var. *montanus*. Calyx often shorter than in that variety and scarcely equalling the mature capsule. Seeds 550-600 microns

long, with 10 low ridges, between every two of which lie rows of about 10 large, quite regular cells.

*Distribution:* western slope of the Sierras, south to Kern County, ascending to altitudes of at least 6000 ft. Type: North Fork, Peckinpah, Fresno County, K. Brandegee in 1911 (Baker Herbarium 11460).

*Specimens examined:*

Oregon: Oregon, A. Dix (Mo.)

California: California, Lemmon (U. S.); Sierra Nevada Mts., Lemmon in 1875 (U. S.); Sierra Valley, Lemmon in 1873 (Gray); Rose Springs, M. H. Gates 215 (U. C.); Clark's Ranch, Gray in 1872 (Gray); Riverton, K. Brandegee in 1908 (U. C.); south fork of American River, Hawihome & Blaisdell in 1899 (Cal. Acad.); Coloma, Palmer 2377 (U. S.); N. Y. Falls, Amador Co., Hansen 38 (Dudley, Mo., U. C., U. S. as no. 98); Amador Co., Greene in 1889 (N. Y., U. C.); Calaveras Co., Greene in 1889 (U. C.); Indian Creek, Drew (U. C.); Mokelumne Hill, Blaisdell (Cal. Acad.); Cherry River, Chestnut & Drew in 1889 (U. C.); Hetch Hetchy Trail, Hall & Babcock 3388 (Mo., N. Y., U. C., U. S.); Rawhide Hill, Mrs. Williamson 115 (Dudley, Cal. Acad., N. Y.); Mt. Bullion, Bolander 4855 (Gray, U. C., U. S.); Wawona, Hall 9010 (U. C.); Mariposa, Congdon in 1882 (Dudley); Mariposa Trail, Mann (Gray); Yosemite, K. Curran in 1883 (Gray); Yosemite, Muir 5492 (Mo.); Yosemite, Lemmon (Mo.); Sequoia Mills, T. S. Brandegee in 1892 (Gray); Peckinpah, K. Brandegee in 1911 (Baker); Pine Ridge, Hall & Chandler 264 (U. C.); Toll House, Fresno Co., Hall & Chandler 11 (Mo., U. C., U. S.); Rush Creek Mill, Mrs. McCardle in 1895 (Cal. Acad.); Centreville, MacNeil in 1896 (Cal. Acad.); Big Tree Grove, Lemmon 423 (Cal. Acad.); Middle Tule River, Purpus 1366 (U. C.) and 5611 (Gray, Mo., U. C., U. S.); Kaweah River, Dudley in 1902 (Dudley); Eshom Valley, Mrs. Clemens in 1910 (Baker); Three Rivers, Coville & Funston 1296 (U. S.); Poso Creek Valley, Dudley 560 (Dudley). The Torrey specimen (N. Y.) from Lake County in 1865 has one plant near this variety.

This variety is practically impossible to distinguish from *montanus* with which it has been classified, unless mature seeds are available, but the absolutely different seeds and the well marked range warrant its recognition as a variety. Some plants, such as Mrs. Clemens' specimen from Eshom Valley, show an intermediate condition between this and *capillaris*, having the seeds and flowers of *interior* and the fruit of *capillaris*.

9. *N. rigidus* var. *capillaris* (Greene) Munz comb. nov.

*N. capillaris* Greene in Bull. Cal. Acad. 1: 196. 1885.

*N. ramosissimus* Nutt. in Gray, Synop. Flora, Suppl. II, 1: 39. 1886, in part.

(Plate X, figs. 48-50)

Larger specimens well branched, smaller ones often very slightly so; stems 5 to 25 cm. long, slightly zigzag, generally fine and capillary, glabrous. Basal leaves glabrous, ovate to spatulate-ovate,  $\frac{1}{2}$  to 2 cm. long. pedicels exceedingly fine, tending to be straight and not sinuous. Calyx tube long turbinate below, longer than the lobes; whole not exceeding 2 mm. Corolla slightly exceeding calyx, about 1 mm. long, divided almost to base. Stamens equaling corolla; appendages much reduced, but with pendent cells. Mature capsule surpassing calyx; seeds few, 5 to 12, 630 microns long, with broad, rounded ridges and 9 to 10 cells per row.

*Distribution*: northern California south along the western slopes of the Sierras to the Mohave desert. Types: Mohave desert, *K. Curran in 1884*, and Lake County (?), *D. Cleveland in 1882*.

*Specimens examined*:

California: Snow Mt., *T. S. Brandegee in 1891* (U. C.); Buck Mt., Humboldt Co., *Tracy 2842* (U. C., U. S.); Eureka-Red Bluff Road, *Abrams 6175* (Dudley); Stony Creek, *Rattan in 1884* (Dudley); Mad River Valley, *Tracy 4323* (U. C., U. S.); Montgomery Creek, *Eastwood in 1912* (Cal. Acad., Gray); Burney Falls, *Eastwood in 1912* (Cal. Acad.); Goose Valley, *Eastwood 788* (Cal. Acad., Gray, N. Y., U. S.); Ukiah, *Eastwood 3373* (Cal. Acad., Gray, Mo., N. Y.); Long Valley, Mendocino Co., *Rattan in 1882* (Dudley, Gray); Elk Mt., Lake Co., *Tracy 2296* (U. C.); Lake Co. (?), *Cleveland in 1882* (Cal. Acad.); Lake Co., *Torrey 281* (Gray, N. Y.), *Torrey in 1865* in part (N. Y.); Lake Co., *Greene 278* (Gray); Butte Creek, *Leiberg 5025* (U. S.); Plumas Co., *Heller 10841* probably this (Gray, Mo., Phila., U. C.); Mt. St. Helena, *Eastwood 4676* (Cal. Acad.); American Valley, *Mrs. Austin in 1879* (U. C.); Cape Horn, Placer Co., *K. Brandegee in 1908* (U. C.); Hams Station, Amador Co., *Hansen 1101* (Dudley); between Cold Spring and Long Barn, Tuolumne Co., *Abrams 4732* (Dudley, Gray); between Yosemite and Big Tree grove, *Lemmon* (U. S.); Santa Clara Co., *Mrs. Ames?* (U. S.); Kern River, *Dudley 801* (Dudley); Mohave desert, *M. K. Curran* or *K. Brandegee in 1884* (Cal. Acad., Gray, U. C.); Colton, *M. E. Jones 3395* in part (Baker, Cal. Acad.).

The exceedingly small flowers and fruits and the elongated base of the calyx make this variety quite easy of determination. It does not seem much like the species *rigidus*, but is connected with it through *interior*. The Eastwood collection at Burney Falls and Mrs. Clemens' collection at Eshom Valley are intermediates with *interior*.

10. *N. rigidus* var. *rubescens* (Greene) Munz comb. nov.

*N. rubescens* Greene in Bull. Cal. Acad. 1: 197. 1885.

*N. ramosissimus* Nutt. in Torrey, Bot. Mex. Bound. Survey, 108, Pl. 35. 1859.

*N. ramosissimus* var. *montanus* in Gray, Synop. Flora, Suppl. II, 1: 393. 1886, in part, and in Coulter, Bot. West Texas, Contr. U. S. Nat. Herb. 2: 250. 1892.

*N. adenophorus* Parish, Bull. S. Calif. Acad. 2: 28. 1903.

(Plate X, figs. 51-55)

From slightly to diffusely branched, stems fine, 5 to 25 cm. long, scarcely zigzag, mostly glabrous. Basal leaves  $\frac{1}{2}$  to 2 cm. long, obovate, entire to pinnatifid, mostly pubescent. Flowers on capillary, mostly recurved, spreading pedicels. Calyx tubular for scarcely  $\frac{1}{3}$  its length, much exceeded by the corolla of almost separate petals. Corolla bilabiate, about 2 mm. long, the three lobes of the upper lip generally ciliate; corolla white or tinged with purple. Stamens well exerted, the appendages with pendent translucent cells. Capsule broad, exceeding or equaling the calyx; seeds about 450 microns long with 10 longitudinal rows of 12-14 weak and irregular cells.

*Distribution:* deserts of California, southern Nevada and Utah, Arizona, New Mexico, and northern Lower California, ascending to elevations of 7000 ft. (Hall & Chandler 6996, Panamints). Type: Reno, Nevada, K. Curran in 1884, and Mohave desert, California, K. Curran in 1884.

*Specimens examined:*

California: California, Bridges 146 (N. Y., U. S.); California, Arizona, etc., Palmer 300 (Gray); Mohave desert, K. Curran in 1884 (Baker, Cal. Acad., U. C.), J. G. Lemmon & Wife in 1884 (U. C., U. S.), S. B. & W. F. Parish 1338A (Gray), Lemmon 3131 and 3132 (Gray), Grant 4373 (N. Y.); Bishop, M. E. Jones in 1897 (Baker); Argus Mts., M. E. Jones in 1897 (Baker); Pleasant Canyon, Panamints, Hall & Chandler 6996 (U. C.); Panamint Mts., Coville & Funston 677 (Gray, Mo., N. Y., Phila., U. S.); Salt Well, Inyo Co., Hall & Chandler 6886 (U. C., Wyo.); White Mts., Inyo Co., Heller in 1906 (Phila.); Little Lake, Inyo Co., Davidson 2422 (Dudley, U. C., U. S.), and Hall & Chandler 7362 (Baker, U. C.); Panamint Valley, Parish 10111, 10146, 10164 (Dudley); Panamint Canyon, M. E. Jones in 1897 (Baker, Mo., U. S., Wyo.); Kramer, K. Brandegee in 1913 (Baker); Kelso, M. E. Jones in 1906 (Baker, U. S.); Lone Willow spring, Parish 10184 (Dudley); Greenwater flat, Parish 9879 (Dudley); Soda Lake, Parish 10010 (Dudley); Needles, Munz & Harwood 3643 (Baker); Goffs, M. E. Jones in 1903 (Baker); Piute Creek, Wilson in 1893 (U. C.); Providence Mts., Munz, Johnston & Harwood 4230 (Baker); Sheep Hole Mts., Hall 6046 (U. C.); Daggett, Hall 6144 (Dudley, U. C., U. S.); Barstow, Parish 9248 (Dudley), K. Brandegee in 1905 (Baker), Mabel Minthorn in 1914 (U. S.); 15 miles north of Victorville, Johnston 6503 (Baker) and Peirson 1209 (Peirson); Victorville, M. E. Jones in 1903 (Baker, Mo.); Rabbit Springs, Parish 4956 (Cal. Acad., Dudley, U. C., U. S., Wyo.); Ord Mts., Hall & Chandler 6819 (U. C.); San Bernardino, S. B. & W. F.

*Parish* 831 in part (U. C., U. S.); Palm Springs, *Mrs. Wilder in 1907* (Dudley), *Eastwood* 2991 (Cal. Acad.), *Mrs. Spencer* 1498 (Baker); Whitewater, *M. E. Jones in 1903* (Baker); Indio Mt., *Hall* 5819 (Baker, Dudley, Gray, Mo., Phila., U. C., U. S.); Shavers well near Mecca, *Munz & Keck* 4745 (Baker); Eagle Mts., *Munz & Keck* 4824 (Baker); Chuckwalla Mts., *Munz & Keck* 4852 (Baker); Salton, *Hall* 5838 (Dudley, U. C., Wyo.); Colorado desert, *T. S. Brandegee in 1905* (U. S.); Borregos Springs, *T. S. Brandegee in 1895* (Baker, U. C.); Yaqui Wells, *Eastwood* 2686 (Cal. Acad.); s. w. part of Colorado desert, *Orcutt in 1889* (Gray, Mo., part of U. S.); Two-bunch Palms, *Jaeger* 441 (U. S.)

Nevada: Reno, *K. Curran in 1884* (Cal. Acad.); Las Vegas, *Goodding* 2319 (Gray, Mo., N. Y., U. C., Wyo.); Good Springs, *M. E. Jones in 1905* (Baker); Mica Springs, *M. E. Jones* 5045 (Baker, U. S.); Maopa, Lincoln Co., *Kennedy* 1079 (U. S.).

Utah: Utah, *Parry* (Cal. Acad.); southern Utah, *Parry* 143 (Gray, Mo., Phila., N. Y.); St. George, *M. E. Jones in 1880* (Baker, Gray).

Arizona: Beaverdam, *Jones* 5024ad (U. S.); Pierces Ferry, *Jones* 5077ae (Baker, U. S.); Peach Springs, *Jones in 1904* (Baker); Yucca, *Jones* 3920 (Baker, U. S., Wyo.); Franconia, *Jones in 1903* (Baker, Mo., U. S.); Chimehuevis, *Jones in 1903* (Baker); Congress Junction, *Jones in 1903* (Baker); Wickenburg, *Jones in 1903* (Baker); Castle Rock, *Toumey* 459 (U. S.); Phoenix, *Eastwood* 6176 (Cal. Acad.); Tucson, *Pringle in 1884* (Phila.), *Toumey* 217 (U. S.); Sabina Canyon, *Miss Zuck* (U. S.); Tucson Mt., *Griffiths* 2425 (N. Y.).

New Mexico: New Mexico, *C. Wright* 1431 (Gray, Phila., U. S.); west of El Paso, *Parry in 1852* (Phila.); valley of Rio Grande below Doñana, *Mex. Bound. Survey* 695 (U. S.).

Lower California: Grulla, *Orcutt in 1886* (U. C.).

This variety is for the most part well marked and seems almost of specific rank. The well exerted stamens and the ciliate condition of the upper corolla lobes are very characteristic, but there are many specimens almost or quite destitute of such ciliation and almost like *interior*. Having so wide a distribution, it is not surprising that there is some variation; for instance, many of those specimens from the Indio region of the Colorado desert have the basal leaves dentate to pinnatifid, and from the Needles and adjoining region come plants with a peculiar erect, unbranched habit and few hairs on the petals in which the veins are few and almost straight. In many such specimens the seeds are narrower than in the typical form and approach in character the low, flat ridges of *montanus*. But in all such cases no constant differentiating characters could be found that seemed worthy of nomenclatorial recognition. Overlapping to some extent the range of *N. ramosissimus* var. *gracilis* and often resembling that variety

in habit, this variety is sometimes distinguishable from it only by the more deeply divided corolla. Varietal rank is given to this desert plant because of the relationship to *montanus* and *interior*, with which plants it certainly belongs in classification; ciliation of corolla is not a specific distinction and shows similarity to *N. rigidus* var. *australis*.

POMONA COLLEGE,  
CLAREMONT, CALIFORNIA

#### EXPLANATION OF PLATES

Floral parts  $\times 12$ ; seeds  $\times 25$ ; leaves  $\times 1$ ; habit  $\times \frac{1}{2}$ .

#### PLATE IX

FIGS. 1-7. *Nemacladus longiflorus* (Parish 7081, U. C. 166790). 1, the flower; 2, basal leaf; 3, seed; 4, corolla, split lengthwise; 5, capsule; 6, habit of stem and pedicels; 7, stamens, showing appendages and two of the ovary glands.

FIGS. 8-16. *N. ramosissimus* (Parish 11266, Baker 4712; the seed, Nuttall, Gray). 11, staminal appendage; 16, ovary glands, with staminal appendages omitted.

FIGS. 17-23. *N. ramosissimus* var. *pinnatifidus* (E. L. Greene in 1895, U. C. 102226).

FIGS. 24-29. *N. ramosissimus* var. *gracilis* (Heller 7729, U. C.; habit from Eastwood type, Cal. Acad.).

#### PLATE X

FIGS. 30-36. *N. rigidus* (K. Curran in 1884, U. C. 110961).

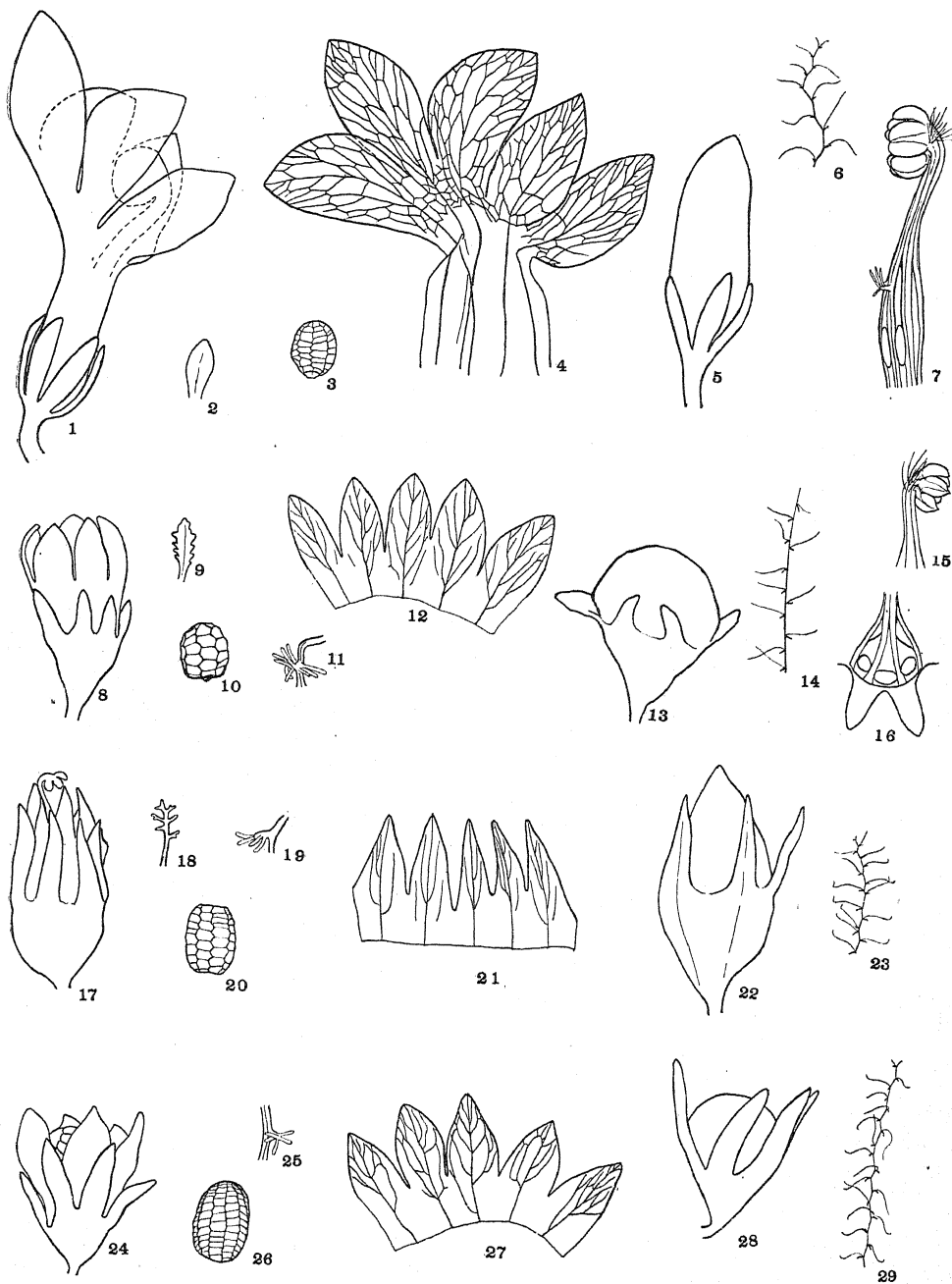
FIGS. 37-40. *N. rigidus* var. *australis* (Orcutt 1348, Gray).

FIGS. 41-46. *N. rigidus* var. *montanus* (Cleveland, Allens Springs in 1882, U. C. 102224).

FIG. 47. *N. rigidus* var. *interior* (K. Brandegee in 1911, Baker 11460).

FIGS. 48-50. *N. rigidus* var. *capillaris* (K. Curran in 1884, U. C. 102221).

FIGS. 51-55. *N. rigidus* var. *rubescens* (K. Curran in 1884, U. C. 102232).



MUNZ: THE GENUS NEMACLADUS







MUNZ: THE GENUS NEMACLADUS



## CYTOLOGICAL STUDIES IN THE GENUS RUBUS<sup>1</sup>

ALBERT E. LONGLEY

(Received for publication July 17, 1923)

A review of the changes in the genus *Rubus* is striking. Twenty-five years ago Gray's "Manual" recognized 5 species and 2 varieties for New England. In 1893, Bailey published his "Evolution of Native Fruits," in which he listed 13 species of *Rubus* of the *Eubatus* group for New England. In the next ten years Blanchard published detailed descriptions of 40 more forms, most of which he ranked as species. Bicknell (7) makes the following remark concerning Blanchard's species:

Probably 60 percent of these are synonyms, while the remainder, with possibly a few exceptions, appear to disclose themselves as scarcely doubtful hybrids.

In his opinion there are about 11 species of *Eubatus*. Through crossing he sees a possibility of 45 primary hybrids, and he says that it appears as if nearly all of these existed. Rydberg (62), in his monograph of the North American blackberries, recognized 27 species for the northeastern United States, including 11 recently described by Blanchard or Bailey, while 24 of Blanchard's species he ranks as hybrids. In 1921, Brainerd and Peitersen (9) described 12 species and 14 hybrids with their type localities, and in addition they give a list of 32 local hybrids.

This phenomenon of variability is not confined to New England *Rubi* alone. Hooker, in his "Flora of the British Isles," names 41 species, while Rogers ("Handbook of British *Rubi*," 1900) lists 100 species. Garke's "Flora von Deutschland" contains more than 60 species for all Europe, while Täckholm (69), referring to Focke's work on *Rubus*, makes the following remark:

Bei keiner andern Untergattung (*Eubati*) der *Rubi* begegnet man einer solchen Vielgestaltigkeit. Hunderte oder Tausende lokaler Kleinarten sind beschrieben worden.

The lack of agreement in the above-cited papers suffices, perhaps, to indicate the difficulties of applying the ordinary methods of classification to such a variable genus. The experimental plant breeder has recently turned to investigate the genus *Rubus*. Lidforss left a posthumous paper (47) in which he draws a pointed parallel between artificially produced hybrids and the variable *Rubi* found in the field. More recently, Brainerd and Peitersen (9) and Peitersen (55) report the efforts to untangle the puzzling condition existing in our local blackberries by experimental breeding. This work has shown that two major factors are responsible for variations found in North American blackberries: (1) Changes due to environ-

<sup>1</sup> Contribution from the Laboratories of Plant Morphology of Harvard University.

mental conditions as shade, temperature, etc. (2) Changes resulting from the propagation of heterozygous forms.

Lidforss and Peitersen have shown the genetical impurity of many of the variable forms of blackberries by crossing inter-fertile species. The hybrids thus produced behave in every way like many forms found growing in the wild, and the conclusion is that such wild forms are heterozygous due to a hybrid origin.

We ask, how are we to know heterozygous forms from pure species? Genetically impure species are characterized by gigantism, variability, reproductive sterility, polyploidy, and polyspory. The presence of the first two factors has been demonstrated by experimental work for some of our New England species. Hoar (35) and more recently Peitersen (55) have shown that very few of our native blackberries have morphologically good pollen. Strasburger (66) studied the pollen mother cells in *Rubus leucodermis* Dougl., *R. fruticosus* L., and *R. biflorus* Buch. and found in every case the haploid chromosome number to be 6.

The present work has been undertaken to determine if polyploidy and polyspory exist in the more variable species of this genus.

#### MATERIAL AND METHODS

The material used was collected from labeled material in the plots given over to this genus at the Arnold Arboretum of Harvard University.

The buds were collected on warm days, cut open, and put immediately into a weak chromo-acetic killing fluid. To hasten penetration of the killing fluid, an exhaust pump was used in the field. The buds were imbedded in both paraffin and nitro-cellulose; the latter proved to be the more satisfactory. Several stains were used, but Heidenhain's iron-alum haematoxylin gave the best results.

The material was examined to get as many stages of the pollen-mother-cell development as possible, using a 1.5-mm. Zeiss apochromatic objective and no. 12 compensating ocular. The drawings were made from selected typical stages in the pollen development, with care to retain as nearly as possible the normal proportions, using a camera lucida in order to control the proportions.

#### SECTION I

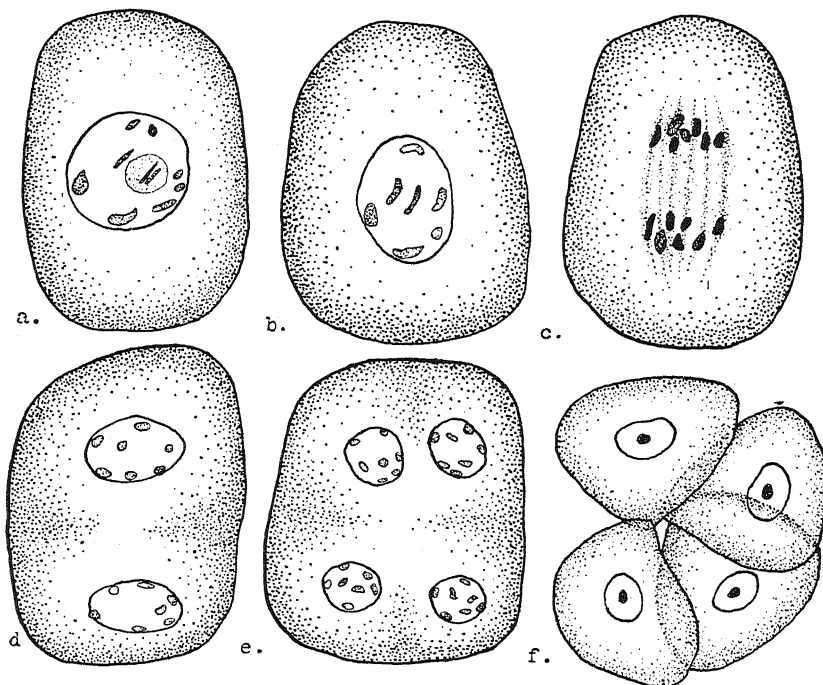
The names used for the Rubi described are taken, as far as possible, from Gray's Manual (56) or from Focke's monograph of the genus *Rubus* (23). I have added also synonyms from Brainerd and Peitersen's (9) recent work on blackberries, from Rydberg (62), and from Bicknell (7).

#### Diploid Rubi

IDEOBATUS FOCKE

*Rubus idaeus* L., var. *canadensis* Richardson. Frankl. Jour. 739. 1823.  
*R. subarcticus* (Greene) Rydberg.

Some stages of the development of the pollen mother cells are shown in text figure 1. In the heterotypic prophase the chromosomes appear at first unpaired, but soon become paired; figure 1a represents an intermediate stage where some are still unpaired and others are distinctly bivalent.



TEXT FIG. 1. Pollen-development of *R. idaeus* L., var. *canadensis*: a, b, early and late heterotypic prophases; c, heterotypic anaphase; d, interkinesis; e, late homoeotypic telophase.

Figure 1b pictures seven chromosomes in a typical diakinesis. The remaining figures show the regularity in the reduction process of this species. In the heterotypic anaphase the chromosomes move as two groups to their respective poles, and the telophases of both the heterotypic and the homoeotypic divisions show clearly the presence of seven chromosomes. The result of this regular meiosis is an equal distribution of the chromatin material to the four nuclei of the tetrad.

*R. occidentalis* L. Sp. Pl. 493. 1753.

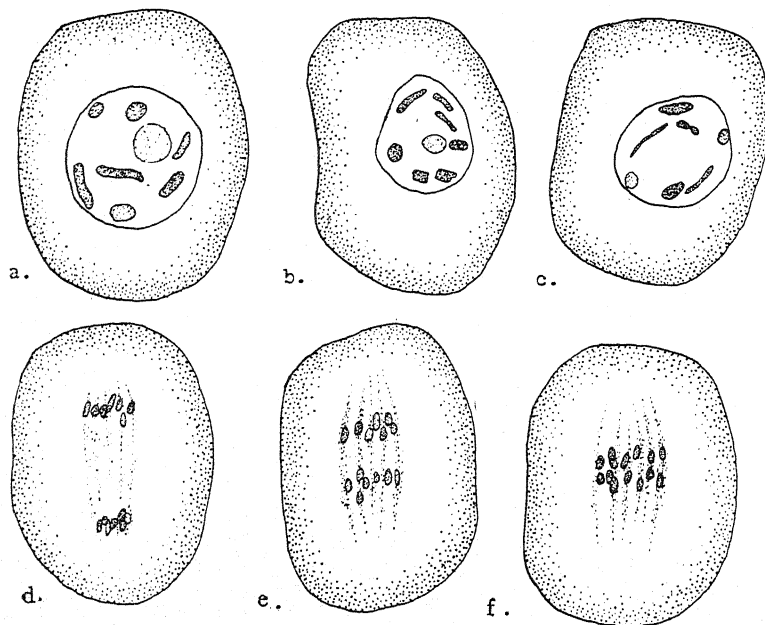
Pollen: a large percent good (Hoar, 35).

The development of the pollen mother cell of this species is represented in Plate XI. Figure 1 pictures the heterotypic prophase before the chromosomes have paired. 14 of these univalents can be distinctly seen, while the nucleolus has become much less prominent. Figure 2 represents the next stage in development, diakinesis, when the bivalent chromosomes stand out very distinctly and the nucleolus has disappeared. The next

step in development is the movement of the chromosomes to the center of the cell. Figure 3 pictures the seven chromosomes arranged on the nuclear plate. Figures 4 and 5 represent two stages of the anaphase, the chromosomes moving in a regular manner towards the poles. Figure 6 represents the heterotypic telophase; each nucleus has seven chromosomes in the periphery. The homoeotypic division is carried out in a perfectly regular manner. Figure 7 pictures the chromosomes moving towards the nuclear plate, and figure 8 the chromosomes moving from the nuclear plate. The homoeotypic telophase (fig. 9) shows the four daughter nuclei, each with its full complement of chromosomes, *i.e.*, 7. From such a regular reduction process four equal pollen grains result, which normally constitute morphologically good pollen.

*R. illecebrosus* Focke. Abh. Nat. Ver. Bremen 16: 278. 1899.

This is an introduced species. A cytological study at meiosis shows it to be a true diploid species. Text figure 2*b* pictures the diakinesis, the nucleolus still faintly persisting. Text figure 2*f* shows the regular movement of the two groups of 7 chromosomes towards the poles.



TEXT FIG. 2. Heterotypic phases: *a* and *e*, *R. odoratus*; *b* and *f*, *R. illecebrosus*; *c* and *d*, *R. frondosus*.

*R. triphyllus* Thunb. Fl. Jap. 215. 1784.

Text figure 3*e* represents the heterotypic telophase with seven chromosomes in each daughter nucleus.

*R. neglectus* Peck. Rep. Reg. N. Y. Univ. 22: 53.



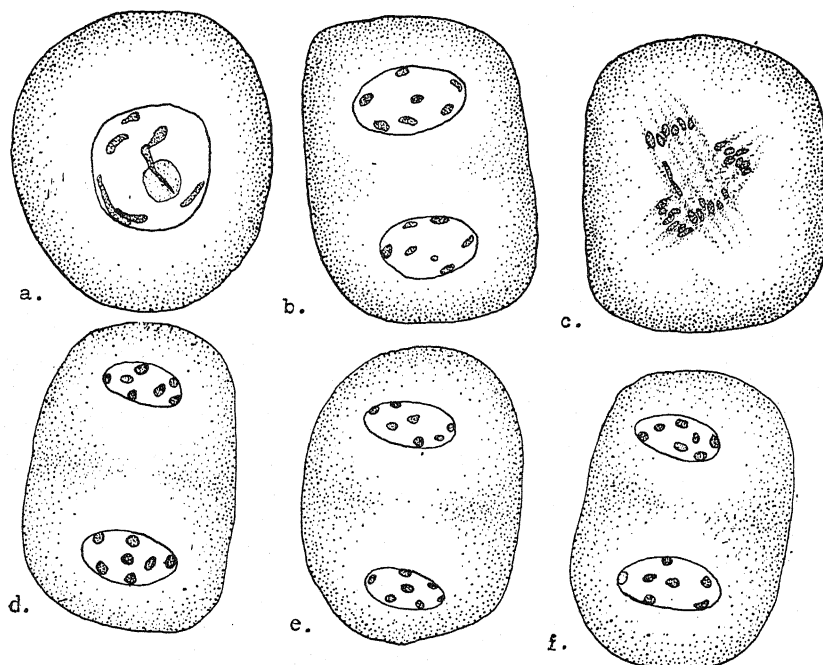
*R. idaeus* × *strigosus*. Focke (23).

*R. occidentalis* × *idaeus* var. *aculeatissimus* (?). (56).

*R. occidentalis* × *strigosus*. Rydberg (62).

Pollen: a large percent imperfect (Hoar, 35).

The chromosome number in this hybrid *Rubus* is distinctly seven. In text figure 3 are pictured three stages in the pollen-mother-cell development. Diakinesis and telophase of the heterotypic division appear in every way regular. An occasional irregularity is pictured in *c* where one chromo-



TEXT FIG. 3. Pollen mother cells: *a-c*, stages in *R. neglectus*, heterotypic prophase, interkinesis, and homoeotypic anaphase; *d-f*, interkineses of *R. Randii*, *R. triphyllus*, and *R. alleghaniensis*.

some is late in dividing, causing it to lag and the two halves to be on the spindle after the other six have reached the poles. A cytological study has revealed nothing to indicate the hybrid origin of this species, and whatever its parents are, they could hardly be other than diploid species.

#### ANAPLOBATUS FOCKE

*R. odoratus* L. Sp. Pl. 494. 1753.

Pollen: a large percent good (Hoar, 35).

A typical diakinesis of this species shows the size and shape for individual chromosomes in this group of *Rubi*, which is most noticeable in the presence of two long chromosomes (text fig. 2*a*), and these are duplicated in

the diakinesis of every pollen mother cell studied. The heterotypic anaphase (text fig. 2e) is perfectly regular, and all subsequent stages are very similar to those pictured for *R. occidentalis*.

EUBATUS FOCKE

*R. alleghaniensis* Porter. Bull. Torr. Bot. Club 23: 153. 1896.

Pollen: a large percent good (Hoar, 35); 4 percent imperfect (Peitersen, 55).

This is one of the few blackberries with 7 chromosomes, a fact which is significant, for it is also one of the few blackberries with morphologically good pollen. Text figure 3f represents the condition in the interkinesis, the seven chromosomes being distinct at this stage, while the homoeotypic anaphase (text fig. 4e) shows the seven chromosomes passing towards each pole.

*R. frondosus* L. Sp. Pl. 493. 1753.

Pollen: a large percent good (Hoar, 35).

This is one of the three species examined by Strasburger (67) and found to have 6 as the haploid chromosome number in the egg and pollen cells. My study of this form shows that its haploid number is 7, and I have represented in text figure 2c the diakinesis in which the chromosomes stand out most distinctly. The heterotypic anaphase (text fig. 2d) shows the regularity with which the seven split chromosomes go to their respective poles. The whole meiosis duplicates conditions found in all the diploid Rubi.

*R. Randii* (Bailey) Rydb. Britton Man. 497. 1901.

*R. argutus* Link. Focke (23).

*R. canadensis* L., in part, Brainerd and Peitersen (9).

Gray's Manual (56) gives *R. recurvicaulis* Blanchard and *R. argutus* var. as synonyms to *R. Randii*, and it was from a bush labelled *R. recurvicaulis* that my material was collected.

*R. pergratus*  $\times$  *procumbens* = *R. recurvicaulis*. Rydberg (62).

*R. canadensis*  $\times$  *Baileyanus* = *R. recurvicaulis*. Bicknell (7).

*R. recurvicaulis*, doubtful status. Brainerd and Peitersen (9).

I was puzzled by the findings in my cytological studies of this form, but after looking up its synonymy there seemed to be the possibility that it was a species ranking with the last two described. Text figures 3d and 4d are drawn from clear kineses, and there seems to be no doubt that the haploid number is 7. It would be of great interest to know the pollen condition of this species, for one would predict nearly perfect pollen in a diploid species behaving so regularly in its reduction divisions.

*R. villosus* (Gray) Ait. in Hort. Kew. ed. 1, 2: 210.

*R. argutus* Link. In part, Brainerd and Peitersen (9).

Pollen: 20 percent imperfect (*R. argutus*; Peitersen, 55).

A study of all the significant stages in the pollen-mother-cell development of this species shows that the chromosome number is 7 and that the

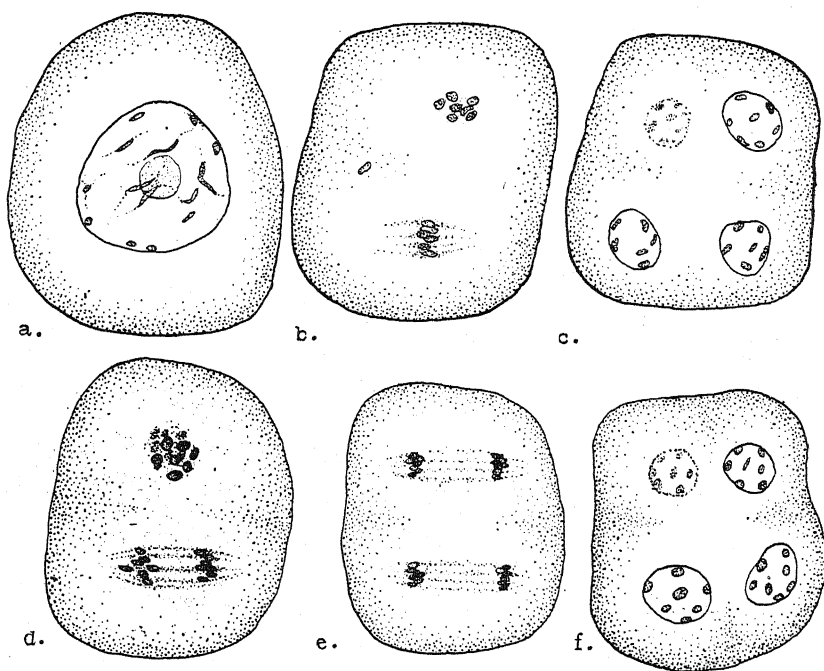
tetrad is developed in a perfectly regular manner. Text figure 4f is a representation of the homoeotypic telophase, each nucleus having received seven chromosomes.

*R. setosus* var.

This form I give as it was labeled at the Arboretum.

Pollen: large percent good (Hoar, 35).

This is the only 7-chromosome *Rubus* in which I have noticed an irregularity occurring at all frequently, even though it is not the rule. There are cases in which a chromosome lags behind in the anaphases of the heterotypic and homoeotypic divisions. Text figure 4 pictures three stages in the reduction division. 4a shows an early prophase before pairing of the



TEXT FIG. 4. Pollen-development: *a-c*, heterotypic prophase, homoeotypic metaphase showing an extruded chromosome on the left, and late homoeotypic telophase of *R. setosus* var.; *d*, homoeotypic anaphase of *R. Randii*; *e*, homoeotypic anaphase of *R. alleghaniensis*; *f*, late homoeotypic telophase of *R. villosus*.

chromosomes occurred. The true diakinesis seems hard to find, and many figures show only part of the pairs fused. Figures 4b and 4c are two stages in which the irregularity referred to earlier is clear, a chromosome extruded into the cytoplasm giving in the tetrad two nuclei with seven chromosomes each and two with only six.

## Tetraploid Rubi

EUBATUS FOCKE

*R. caesius* var. *turkestanicus* Regel. in Gartenfl. 41: 106. 1899.

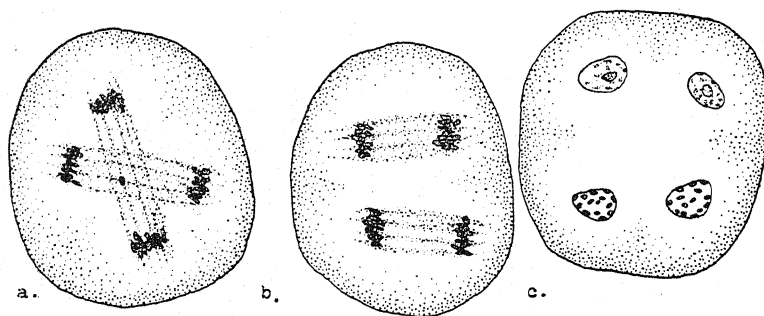
Pollen: Hoar (35) thinks this is not a good species.

Plate XII gives in considerable detail significant stages in the pollen-mother-cell development and also the varying occurrence of a confusing mass (or masses) of dark-staining material in the cytoplasm. This cytoplasmic material varies in visibility, being most prominent at the telophase of each division and lost almost entirely to the view in the metaphase. It is quite general in its occurrence, but I have chosen to show it in this species in which chromosomes do not lag in division and are never extruded into the cytoplasm. As a consequence, stained bodies can not be mistaken for chromosomes or *vice versa*.

Following now through the various stages as they are brought out by the different figures of Plate XII, the first three items represent different stages in the prophase showing the unpaired chromosomes, the pairing of a few, and finally the bivalent chromosomes in diakinesis. It is interesting to note that instead of two long chromosomes there are four, and careful study may show that not only these two chromosomes are duplicated, but that all chromosomes are likewise doubled in this tetraploid species. The anaphases of both heterotypic and homoeotypic divisions are regular, resulting in 14-chromosomed daughter nuclei, as seen in figures 6 and 10, representing the telophases of the heterotypic and homoeotypic divisions. This species differs from previously described species in the fact that in interkinesis the chromosomes lose their identity, a condition unusual in *Rubus*. Sometimes a nucleolus appears. The product of the regular divisions is a normal tetrad, each nucleus having 14 chromosomes and differing in no way from its sister.

*R. corylifolius* Sm. in Sm. Fl. Brit. 542.

Pollen: Hoar (35) gives this as a probably good species.



TEXT FIG. 5. Late stages in the tetrad-formation of *R. corylifolius*: a, homoeotypic anaphase, a chromosome lagging on one spindle; b, typical homoeotypic anaphase; c, homoeotypic telophase.

Although I was unable to get as many stages in the pollen-mother-cell development as I obtained for most species, text figure 5 represents phases in the homoeotypic division. In the telophase the 14 chromosomes can be counted. Figure 5a represents an irregularity rare in this species and in this tetraploid group, *viz.*, a lagging chromosome. Tetrads result normally from an equal distribution of chromatic and cytoplasmic material.

### Triploid Rubi

#### ANAPLOBATUS FOCKE

*R. deliciosus* Torr. in Ann Lyc. Nat. Hist. N. Y. 2: 196. 1828.

Pollen: a good species, judging from its pollen (Hoar, 35).

This is the only *Rubus* outside the *Eubatus* group that had more than seven chromosomes. I was surprised to find this species showing 10 or 11 chromosomes as the haploid number. To make doubly sure, considerable material was cut and it was found that not only in the diakinesis was this number present, but also in the telophases of the heterotypic and homoeotypic divisions the number of chromosomes was about 10, variations occurring as a result of unequal distribution and the extrusion of chromosomes. It was from the early prophase that the diploid number of chromosomes was determined. Since I have made a more detailed study of the triploid Rubi in the *Eubatus* group, I pass on to this subgenus.

#### EUBATUS FOCKE

Conditions are practically identical in the following 13 Rubi, and so I shall review the status of each and then describe in detail the reduction stages found common in triploid forms.

*R. canadensis* L. Sp. Pl. 494. 1753.

Pollen: 25-75 percent imperfect (Hoar, 35); 85 percent imperfect (Peitersen, 55).

*R. setosus* Bigelow. Fl. Bost. ed. 2, 204. 1832.

*R. nigricans*  $\times$  *hispidus*. Bicknell (7).

Pollen: 80 percent imperfect (Peitersen, 55).

*R. sativus* Brainerd. Rhodora 2: 26. 1900.

*R. alleghaniensis*  $\times$  *Baileyanus*. Bicknell (7).

*R. thyrsoides* Wimm. Fl. Schles. ed. 1, 204.

The remaining nine forms have been described by Blanchard, and no monograph ranks them all as species, but practically all have been so treated by different authors.

*R. glandicaulis* Blanchard. Rhodora 8: 172. 1906.

*R. alleghaniensis*  $\times$  *setosus*. Brainerd and Peitersen (9).

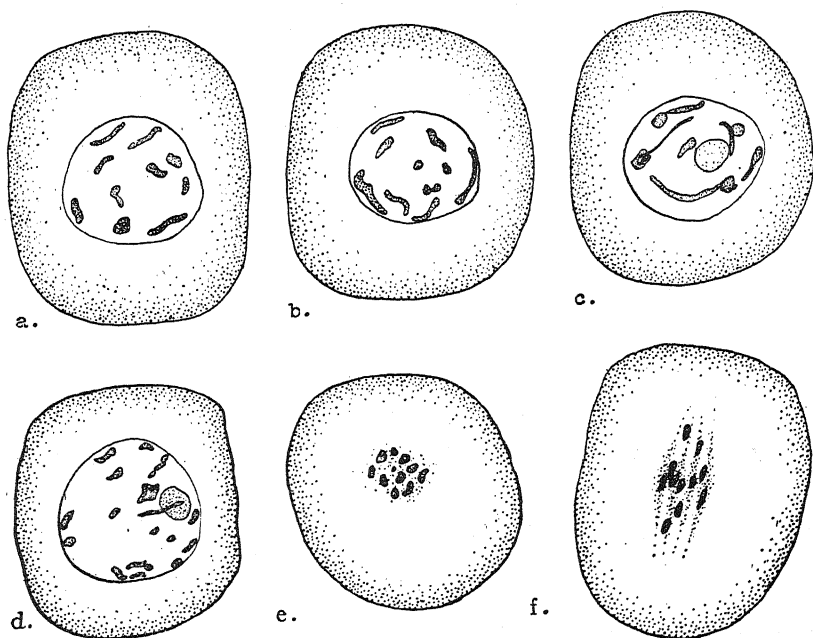
*R. alleghaniensis*. Bicknell (7).

*R. nigricans*  $\times$  *nigrobaccus*. Rydberg (62).

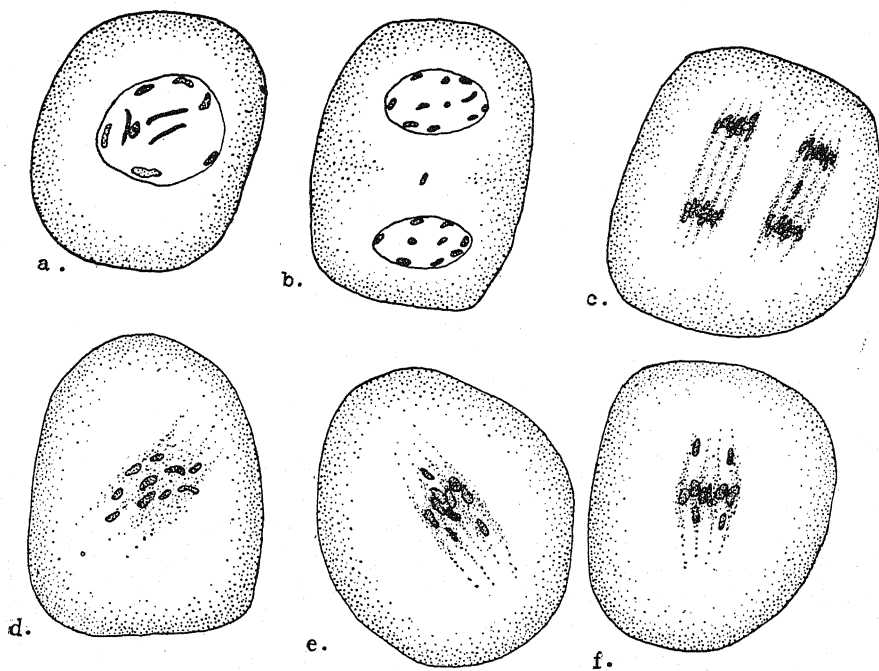
Pollen: 25-75 percent imperfect (Hoar, 35); 60 percent imperfect (Peitersen, 55).

- R. biformispinus* Blanchard. *Rhodora* 8: 178. 1906.  
Of doubtful status. Brainerd and Peitersen (9).  
*R. nigricans*  $\times$  *nigrobaccus*. Rydberg (62).  
Pollen: 75 percent imperfect (Hoar, 35).  
*R. orarius* Blanchard. *Rhodora* 8: 169. 1906.  
*R. pergratus* Blanchard. Gray's Manual (56).  
*R. alleghaniensis*  $\times$  *canadensis*. Brainerd and Peitersen (9).  
*R. alleghaniensis*  $\times$  *canadensis*. Bicknell (7).  
*R. pergratus* Blanchard. Rydberg (62).  
Pollen: 25-75 percent imperfect (Hoar, 35); 94 percent imperfect (Peitersen, 55).  
*R. amnicolus* Blanchard. *Rhodora* 8: 170. 1906.  
*R. pergratus* Blanchard. Gray's Manual (56).  
*R. argutus* Link. Bicknell (7).  
Pollen: large percent good (Hoar, 35); 68 percent imperfect (Peitersen, 55).  
*R. recurvans* Blanchard. *Rhodora* 6: 223-225. 1904.  
*R. frondosus* Bigelow. Brainerd and Peitersen (9).  
*R. frondosus* Bigelow. Bicknell (7).  
Pollen: 25-75 percent imperfect (Hoar, 35).  
*R. Andrewsianus* Blanchard. *Rhodora* 8: 17, 18. 1906.  
*R. alleghaniensis*  $\times$  *argutus*. Brainerd and Peitersen (9).  
*R. argutus* Link. Bicknell (7).  
Pollen: 75 percent imperfect (Hoar, 35); 75 percent imperfect (Peitersen, 55).  
*R. multiformis* Blanchard. *Rhodora* 8: 179. 1906.  
*R. Baileyanus*  $\times$  *canadensis* or *canadensis*  $\times$  *flagellaris* (in part). Brainerd and Peitersen (9).  
*R. canadensis*  $\times$  *hispidus*. Bicknell (7).  
*R. nigricans*  $\times$  *plicatifolius*. Rydberg (62).  
Pollen: large percent imperfect (Hoar, 35).  
*R. peculiaris* Blanchard. *Rhodora* 8: 174. 1906.  
*R. nigricans*  $\times$  *canadensis*. Bicknell (7).  
Pollen: large percent imperfect (Hoar, 35).  
*R. tardatus* Blanchard. *Rhodora* 8: 178. 1906.  
*R. flagellaris*  $\times$  *setosus*. Brainerd and Peitersen (9).  
*R. nigricans* Rydb. Bicknell (7).  
Pollen: 75 percent imperfect (Hoar, 35).

The somatic chromosome number has been determined for *R. glandicaulis* (Pl. XIII, fig. 1) in cells of the developing ovary and found to be 21. It was found that in the early prophase, before pairing had taken place, there was no difficulty in counting the individual chromosomes. The presence of a distinct nucleolus generally signifies that most of the chromosomes are univalent, and Plate XIII, figure 2 pictures such a condition in



TEXT FIG. 6. Early heterotypic phases of triploid forms: a-c, diakinesis of *R. bifor-mispinus*, *R. multiformis*, and *R. sativus*; d, early prophase of *R. recurvans*; e, early metaphase of *R. orarius*; f, early metaphase of *R. amnicolus*.



TEXT FIG. 7. a-c, stages in the pollen-development of *R. setosus* Bigelow: diakinesis, interkinesis (one chromosome in the cytoplasm), and homoeotypic anaphase. d-f, heterotypic metaphases of *R. thyrsoides*, *R. Andrewsianus*, and *R. peculiaris*.

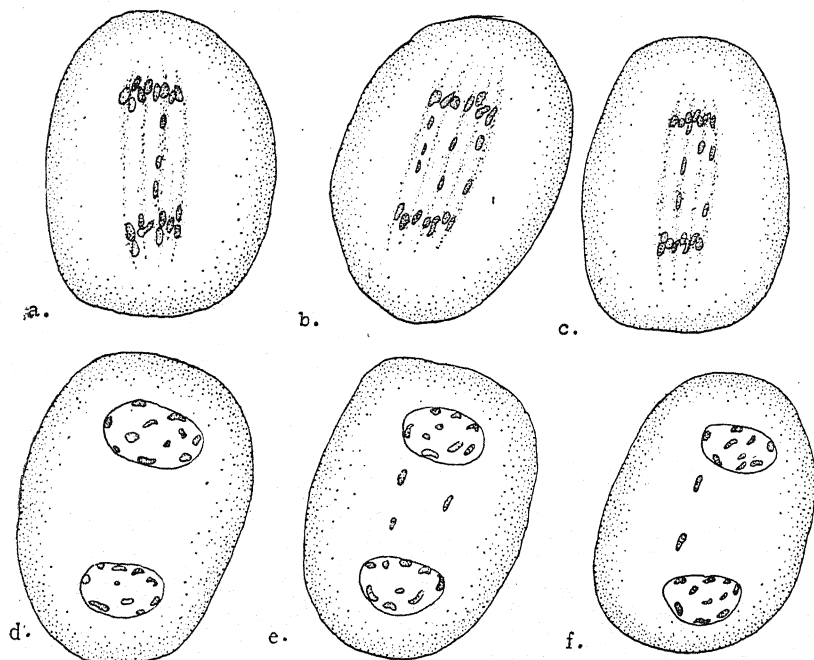


*R. glandicaulis*, while text figure 6*d* represents a very similar condition in *R. recurvans*, one chromosome only being bivalent.

It is with considerable difficulty that one finds a diakinesis in which all the chromosomes are paired, but as a general rule they do pair before going into the nuclear-plate stage; this condition I have shown for *R. glandicaulis* (Pl. XIII, fig. 3), for *R. biformispinus*, for *R. multififormis*, for *R. sativus* and for *R. setosus* (Bigelow) (text fig. 6*a*, *b*, and *c*; text fig. 7*a*). The number of bivalents in all cases was found to be 10, a number that for some time was puzzling until it became apparent that one of these large chromosomes is seemingly formed from a union of three. Later figures will show that often on the spindle are three chromosomes longitudinally in series as if they had been derived from one chromosome.

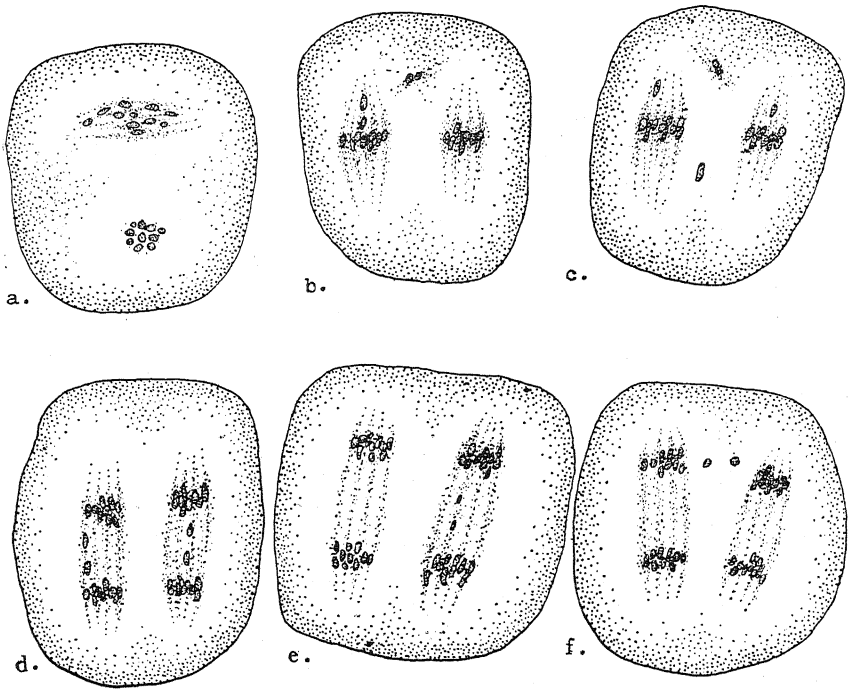
Text figures 7*d*, *e*, and *f* group together three typical metaphases in which the chromosomes are coming to the nuclear plate in a more or less straggling manner. Text figures 6*e* and *f* show two views of the heterotypic metaphase for *R. orarius* and *R. amnicolus*. A condition which is first noticed at this stage and apparent in subsequent stages is that seven chromosomes stand out strongly in consequence of their greater size.

The anaphase is the significant stage in the development of the pollen mother cells of the triploid Rubi. It is in the anaphase that the nature of



TEXT FIG. 8. Critical phases in the heterotypic division: *a*—*c*, anaphases in *R. multififormis*, *R. canadensis*, and *R. tardatus*; *d*—*f*, interkineses in *R. peculiaris*, *R. Andrewsianus*, and *R. biformispinus*.

the irregularities common to this group of blackberries can be analyzed. Seven chromosomes advance to each pole; the remaining three split up into seven smaller chromosomes. Plate XIII, figure 6 shows all seven of these latter chromosomes lagging on the spindle. This condition is very common, and whenever the early anaphase is under observation the seven chromosomes are likely to be found lagging on the spindle. Text figure 8*b* represents an early anaphase of *R. canadensis*, while *a* and *c* are later stages of *R. multiflorus* and *R. tardatus* showing some of the small chromosomes on the spindle. Frequently three are so arranged that I am compelled to conclude they are from a single chromosome.



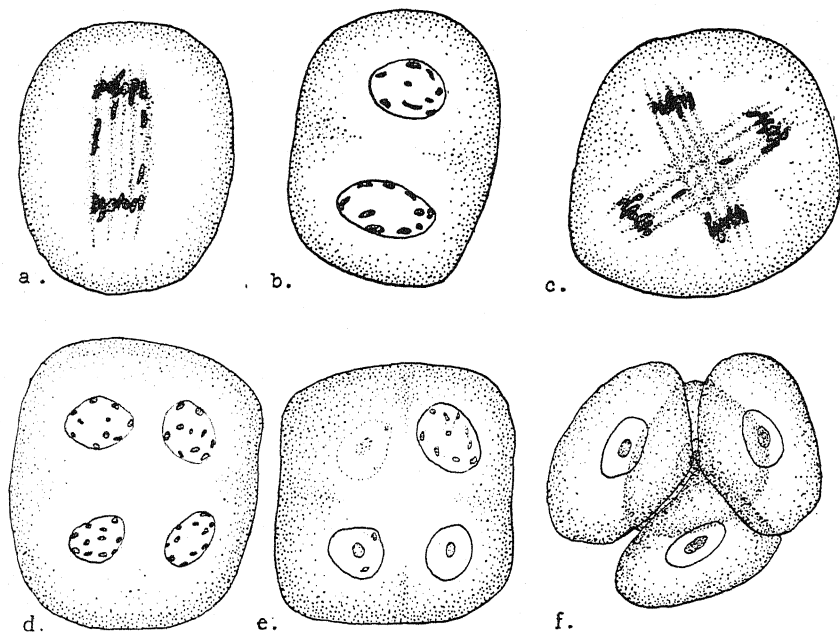
TEXT FIG. 9. Homoeotypic phases: *a-c*, metaphases in *R. thyrsoides*, *R. sativus*, and *R. multiflorus*; *d-f*, anaphases in *R. canadensis*, *R. Andrewsianus*, and *R. biflorus*.

In the telophase there comes an opportunity to check up the number of chromosomes. At this stage they are found in the periphery of the nucleus widely separated. Figures 7 and 10, Plate XIII, are two telophases of *R. glandicaulis*, the former regular with 10 and 11 chromosomes respectively in each daughter nucleus, while the latter shows two chromosomes in the cytoplasm and consequently a loss of two from the resulting daughter nuclei. Text figures 8*d*, *e*, and *f* represent conditions in *R. peculiaris*, *R. Andrewsianus*, and *R. biflorus*. The extrusion of chromosomes, though occurring quite frequently, is not the general situation, and so the

daughter nuclei receive 10 and 11 chromosomes, but they may number from 7 to 12 chromosomes.

Passing now to the homoeotypic division, regular metaphases are seen for *R. glandicaulis* in Plate XIII, figure 8, and for *R. thyrsoides* in text figure 9a. The irregularities found at this stage are seen in Plate XIII, figure 11 and in text figures 9b and c; sometimes the chromosomes found in the cytoplasm have about them a dwarf spindle, but often the excluded chromosomes seem to be deteriorating in the cytoplasm.

The anaphase in the homoeotypic division is generally regular, as I have pictured for *R. glandicaulis* (Pl. XIII, fig. 9), and the occasional irregularities are represented in text figures 9d-f, 10c, and 7c. The irregularities are in the form of lagging chromosomes either on the spindle or in the cytoplasm, extruded during the heterotypic division (fig. 9f). I have never found these isolated chromosomes forming dwarf nuclei and dwarf pollen grains, but it is quite likely they do in rare cases.



TEXT FIG. 10.<sup>7</sup> Stages in pollen-mother-cell development: a, heterotypic anaphase of *R. recurvans*; b, interkinesis of *R. amnicolus*; c, homoeotypic anaphase of *R. orarius*; d, late homoeotypic telophase of *R. thyrsoides*; e, *R. tardatus* just before cell-wall formation; f, tetrad of *R. biformispinus*.

The four nuclei of the telophase have a varying number of chromosomes due to the extrusion of a few of these in the heterotypic and homoeotypic divisions. The extruded chromatin material generally deteriorates before the tetrad breaks up into pollen grains, though it may persist in the form of small black bodies.

The significant points brought out in the development of the tetrads of triploid Rubi are the reduction to 10 bivalent chromosomes, the lagging of 3 of these in the heterotypic anaphase, and the varying number of chromosomes found in the nuclei of the daughter cells. I shall show in later sections of this article the relation of these features to the cytological investigations of other investigators and draw, where possible, appropriate conclusions.

### Pentaploid Rubi

EUBATUS FOCKE

*R. plicatifolius* Blanchard. Rhodora 8: 18. 1906.

*R. flagellaris* × *frondosus*. Brainerd and Peitersen (9).

*R. nigricans* × *procumbens*. Bicknell (7).

*R. villosus* Ait. Rydberg (62).

Pollen: a large percent imperfect (Hoar, 35); 64 percent imperfect (Peitersen, 55).

Plate XIV pictures the pollen development of this polyploid form. The chromosome count is hard to determine with certainty, but after a study of very late prophase conditions I found the number of paired chromosomes to be 17, and, as a consequence of my study of triploid Rubi, I have assumed that this is a pentaploid form. In the metaphase, chromosomes are late in reaching the plate. My material shows very few heterotypic anaphases, but I have pictured an early and a late condition in which the separation of the chromosomes is irregular, and, judging from the presence of extruded chromosomes in all later stages, it seems to be a general rule that one, two, or more chromosomes are shut out in the formation of the two daughter nuclei. Figure 6 represents such a condition where 4 chromosomes are in the cytoplasm, and it will be seen that the nuclei lack these four chromosomes to make up the full complement in the two daughter nuclei, *i.e.*, 35.

Early metaphase of the homoeotypic division shows the movement of the chromosomes, to form the nuclear plate, to be regular. Chromosomes are also present in the cytoplasm, left there from the previous division. Figure 8 represents the late metaphase; besides the two major groups, some of the isolated chromosomes on the left are forming a dwarf spindle. Figure 9 shows a little later condition, and in figure 10 the separation of the chromosomes is nearly complete. There is generally less lagging in the homoeotypic anaphase than in the heterotypic anaphase, and the figure exemplifies this. Figure 11 pictures the four major nuclei in the telophase of the homoeotypic division, and in addition two dwarf nuclei and two isolated chromosomes. The result of this irregular distribution of chromosomes is the variation found in the chromosome number of the daughter nuclei, as seen in the early homoeotypic telophase, and the consequent variation in the chromosome number of the pollen grains. Secondly,

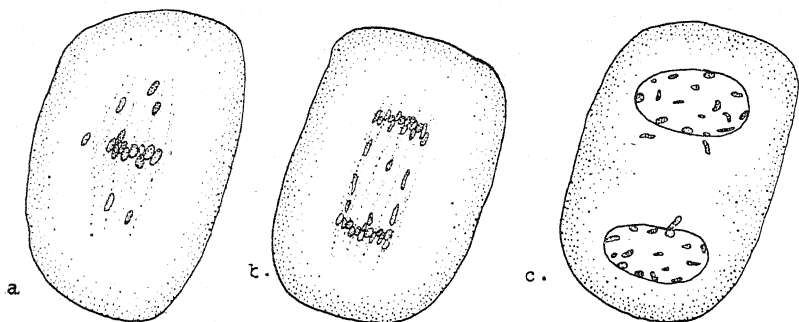
dwarf nuclei make their appearance and dwarf pollen grains are quite general.

A variation in the number of chromosomes in the pollen grains is very common in all the forms studied except the diploid and tetraploid. The appearance of dwarf grains is, however, rare, and I have seen very few indications of these, though they do occur in forms where a large number of chromosomes are extruded, *viz.*, in the triploid and particularly in the pentaploid forms.

*R. hispidus* L. Sp. Pl. 493. 1753.

Pollen: 90 percent imperfect (Peitersen, 55).

Text figures 11a, b, and c represent three stages in the heterotypic division of this species, showing irregularities much as are found in the triploid forms. The chromosome counts apparently justify the conclusion that this is a pentaploid form. Telophases of the heterotypic division show varying chromosome numbers, but these are never more than seventeen in the many counts I have made.



TEXT FIG. 11. Early stages in the pollen-development of *R. hispidus*: a, heterotypic metaphase; b, heterotypic anaphase; c, interkinesis.

### Hexaploid Rubi

EUBATUS FOCKE

Here there is great difficulty in distinguishing paired from unpaired chromosomes in the heterotypic prophase, and so it is not always safe to say the haploid number is 21, but from a study of the telophases of both heterotypic and homoeotypic divisions I have found the number so near 21 that, allowing for the extrusion of a few chromosomes, I feel certain the forms listed below are hexaploid.

*R. arundelanus* Blanchard. *Rhodora* 8: 176. 1906.

*R. frondosus* Bigelow. Brainerd and Peitersen (9).

*R. recurvans* Blanchard. Rydberg (62).

Pollen: 25-75 percent imperfect (Hoar, 35); 74 percent imperfect (*R. frondosus*; Peitersen, 55).

*R. frondosus* Bigelow. Fl. Bost. ed. 2, 199. 1824.

Pollen: 75 percent imperfect (Hoar, 35); 75 percent imperfect (Peitersen, 55).

*R. Jeckylanus* Blanchard. *Rhodora* 8: 177. 1906.

*R. argutus*  $\times$  *frondosus*. Brainerd and Peitersen (9).

*R. alleghaniensis*  $\times$  *Baileyanus*. Bicknell (7).

*R. recurvans* Blanchard. Rydberg (62).

Pollen: 25-75 percent imperfect (Hoar, 35); 60 percent imperfect (Peitersen, 55).

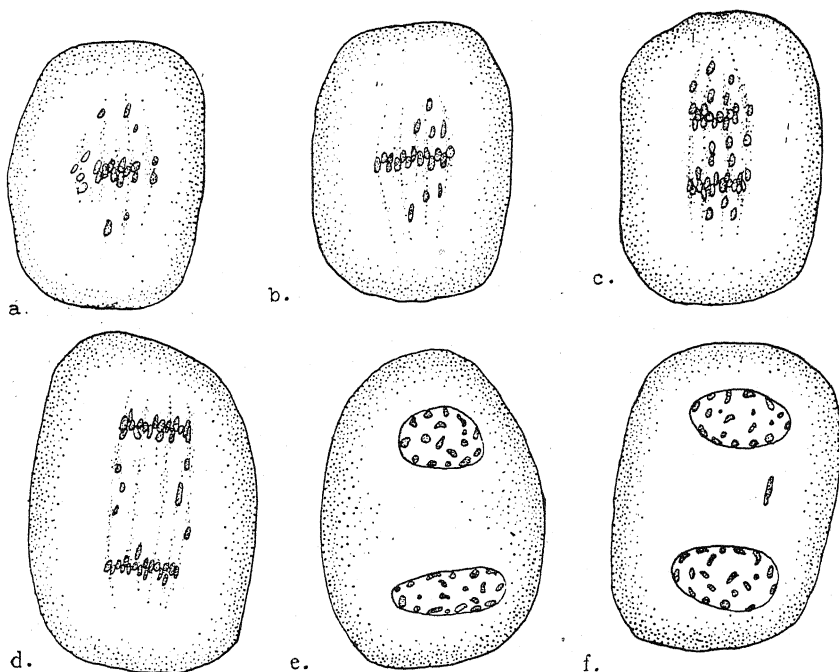
*R. semierectus* Blanchard. *Rhodora* 8: 157. 1906.

Of doubtful status. Brainerd and Peitersen (9).

*R. nigricans*  $\times$  *procumbens*. Bicknell (7).

*R. hispidus*  $\times$  *plicatifolius*. Rydberg (62).

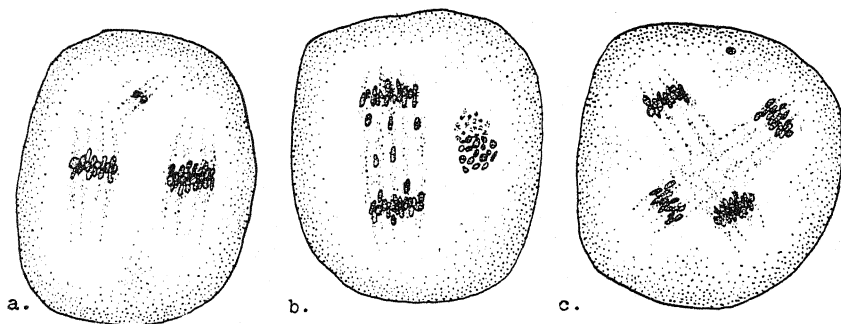
Pollen: large percent imperfect (Hoar, 35).



TEXT FIG. 12. Early stages in the pollen-development of four hexaploid forms: *a*, *b*, heterotypic metaphases of *R. arundelanus* and *R. Jeckylanus*; *c*, *d*, heterotypic anaphases of *R. semierectus* and *R. frondosus*; *e*, *f*, interkineses of *R. frondosus* and *R. Jeckylanus*.

The individual variations in the four forms just cited are slight, and so I shall point out here and there a few of the outstanding cytological conditions during meiosis. I have not pictured a diakinesis, for they are hard to find and it is hard to give a true interpretation of those discovered. The large number of chromosomes and the slowness of their pairing lead to extreme confusion. Text figure 12*a* represents the metaphase of *R. arunde-*

*lanus* where a large number of the chromosomes are scattered on the spindle after the nuclear plate has been formed. Text figure 12*b* illustrates a similar irregularity in the heterotypic metaphase of *R. Jeckylanus*. Text figure 12*c* represents an early anaphase of *R. semierectus*. Here two groups are moving towards the poles and, in addition, there are laggards distributed on the spindle. Text figure 12*d* shows the separation of the chromosomes nearly complete in *R. frondosus* with laggards appearing much as they do in the triploid Rubi. Text figures 12*e* and *f* represent interkineses of *R. frondosus* and *R. Jeckylanus*, the latter showing one chromosome in the cytoplasm. The count of the chromosomes indicates that several have



TEXT FIG. 13. Stages in the homoeotypic division of three hexaploid forms: *a*, *R. semierectus*; *b*, *R. arundelanus*; *c*, *R. Jeckylanus*.

been extruded, if it be a true hexaploid *Rubus*. Text figure 13*a*, of *R. semierectus*, shows, in addition to the major groups of chromosomes, a small cluster of chromosomes in the upper part of the figure, which is likewise in the process of forming a spindle. Text figure 13*c*, of *R. Jeckylanus*, shows a stage where no irregularity was observed, but in the cytoplasm appears an indication of a chromosome left over from the heterotypic division.

### Octoploid *Rubus*

EUBATUS FOCKE

#### *Rubus* ?

This form was collected at the Arnold Arboretum on a sunny hillside. I took it to be *R. hispidus*, but will call it only a *hispidus*-like *Rubus*. It is common at the Arboretum and probably elsewhere.

Plate XV gives in detail the development of the pollen mother cell. Beginning with the diakinesis, there are rare cases where the chromosomes can be counted. I do not hesitate to state that there are as many as 28 haploid chromosomes, a conclusion which is reached by the study of telophases of both the heterotypic and the homoeotypic divisions. It is only seldom that 28 chromosomes can be counted; there are, however, irregularities in the distribution of the chromosomes, and it is to these extruded chromosomes that I attribute the variation in chromosome number in



nuclei studied. I do not find polyspory here, and the extruded chromosomes do not seem to group together but degenerate in the cytoplasm.

The significance of these cytological findings will be discussed in the last section of this article.

## SECTION II

Die Kenntniss der Bastarde zwischen verschiedenen Thierarten, insbesondere zwischen Pferd und Esel, reicht bis ins Alterthum zurück.

In this manner Focke (22) opens Part II of his "Pflanzenmischlinge." He reviews in an able manner the history of artificially produced hybrid plants and points out that our knowledge of these goes back hardly two centuries.

The discovery of sex organs in the higher plants at the close of the seventeenth century opened a very fertile field for research, both economic and scientific. Gardeners began to recognize hybrids springing up in their gardens, but the first authentic record of such a hybrid was in the year 1694. In that year Camerarius (11) in a letter to Valentinus mentions the effect of crossing the hemp (*Cannabis*) with the hop (*Humulus*). A cross between *Dianthus Caryophyllus* and *D. barbatus* dates from 1719, and this hybrid is still known under the name of Fairchild's sweet william.

It was Linnaeus who first suggested the rôle hybrids might play in increasing the number of plant species. This idea is brought out in such names as *Campanula hybrida* L., *Trifolium hybridum* L., *Trigonella hybrida* L., etc., and he even crossed in his garden species of *Verbascum*, *Mirabilis*, and *Tragopogon*.

Kölreuter (44) is recognized as the pioneer plant hybridizer. His work dates from 1761, when he reported on a cross between *Nicotiana rustica* and *N. paniculata*. Two characteristics of this hybrid, decreased fertility and increased vegetative vigor, have stood in the foreground of reports of subsequent plant breeders, and at the present day these factors confront the geneticist in his experimental research.

The works of Knight (43) and Herbert (34) have attracted much attention; the former was of the opinion that sterility is the result of hybridization. Herbert's idea of a hybrid was much broader than that of Knight and included crosses between nearly related species.

Focke (22) has pointed out that no peculiarity of hybrids has attracted so much attention as the lessening of the power of reproduction, and that crosses between more distantly related plants may even be entirely sterile. Abortive pollen had been recognized as a criterion of hybridization as early as 1832 by Dutrochet (19), who cites as an extreme case *Prunus cerasus* × *P. avium*, in which the "stamina" formed a compact mass in which no pollen was produced. Gärtner (26) also refers to pollen sterility as a criterion of hybridization, and, according to Duchartre (18), Naudin observed that the degree of fertility of hybrids was in direct relation to the number of normal pollen grains.

It is only very recently that the phenomenon of hybrid sterility has been made use of to test species in the wild and so to determine if they were pure or of hybrid ancestry. Oliver (54) states that "natural hybrids have been known for a long time and we have reason to believe that they existed when and where conditions were favorable for their production." Meehan found that cultivated *Viola* produces very few potent seeds, but it was Brainerd (10) who extended Meehan's work to *Viola* in the field and found there many sterile forms. Some hybrids between closely related species are fertile, and it is this class he calls "the despair of the systematist." His experimental work on several species of *Aquilegia*, which he grew indiscriminately together, is worthy of note.

Forsyth (24) studied some *Onagraceae* and found that reduced fecundity is valuable evidence of hybrid derivation, while a uniform development of the microspores is, other things being equal, equally indicative of uncontaminated parentage.

Miss Cole (13) made a study of some of our American roses and found that a large number are characterized by abortive pollen which she thinks is obviously a result of hybridization. Täckholm (69) and Blackburn and Harrison (8) have studied in detail a large number of European roses. They find that variability and sterility characterize the roses of the north temperate zone.

The genus *Rubus* has been monographed by Focke (23) and Sudre (68). Both works describe many hybrids, and sterility is reported as associated with these forms. Hoar (35) made a detailed study of the pollen condition of a large number of species of this genus and found very few that showed good pollen. More recently Lidforss (47), in a morphological and genetical study of many *Rubi*, found much evidence supporting the view that they hybridize in nature and that fertile hybrids reproduce themselves, but the offspring show great variability. Quite recently, in a bulletin (9) from the Vermont Agricultural Experimental Station, is reported work very similar to that of Lidforss but on a larger scale. Peitersen finds that sterility in blackberries is due to defective pollen. The percentage of infertility in a given species is quite constant, and, in view of the experimental and morphological data he presents, he concludes that spontaneous hybridization is quite common in the *Eubatus* group and that a large number of the so-called species are either hybrids or crypthybrids. Jeffrey (38) first described this latter baffling class of hybrids.

The discovery of the law of hybrid segregation by Mendel (52) opened a new era in the history of plant breeders. His work has been of great service to the experimental breeder, especially when dealing with unit characters. When multiple characters are involved, segregation becomes rare and external variability marks the offspring. No one will discount the work of Mendel, but, in consequence of the sterility and variability of hybrids, conditions become very complex. The difficulty of finding geneti-

cally pure species and of distinguishing them from variable heterozygous ones, in forms growing in the wild, has been pointed out by Lotsy (50), in his criticism of experimental work based on animals taken from the wild. However, the variability of the offspring of multiple-factor crosses is of use in determining the heterozygous nature of any particular form, and on this basis we can pretty safely assume that many of the Rubi are strikingly heterozygous and have resulted from multiple-factor crosses. This view is substantiated by the work of Lidforss (47) and Peitersen (55) for the genus *Rubus*. The former author found that the offspring of hybrids are variable, and in these variable offspring it is very interesting to note that he got *gigas*, *semigigas*, *nanella*, and other forms parallel to the *Oenothera* mutants of de Vries.

It is the purpose now to review the cytological investigations where irregularities in the pollen-development have been reported. Rosenberg (57) has pointed out that hybrids have a typical chromosome distribution during the formation of germ cells. After a review of the cytological investigations of hybrids or possible hybrids, I shall endeavor to show that the development of the pollen mother cells of many Rubi is clearly of the type found in hybrids.

The development of pollen tetrads of *Hemerocallis fulva* was shown to have marked irregularities by Strasburger (66). He found that one pollen mother cell frequently develops into four large pollen grains and one, two, or even five dwarf grains. He also refers to like conditions found by earlier authors which he attributes to the same abnormal chromosome distribution.

Ein Bild von Hofmeister welches zwei grössere und einen kleineren Kern in einer Pollenmutterzelle von *Passiflora coerulea*, scheint für eine ähnliche Möglichkeit dort zu sprechen. Vielleicht auch ein Bild mit mehreren Theilstücken innerhalb einer Pollenmutterzelle, das er für *Iris pumila* entwirft. So könnte endlich auch eine Angabe von Wimmel für Fuchsia, wo "die Zahl der in der Pollenmutterzelle entstehenden Theil nicht bestimmt," die Theile auch nicht von gleicher Grösse sein sollen, für eine ähnliche Erscheinung dort sprechen.

Juel (39) studied in detail the stages in the development of the pollen mother cell of *Hemerocallis fulva* and in general confirmed the earlier report of Strasburger, but finds that some of the chromosomes left in the cytoplasm at the heterotypic anaphase degenerate and play no part in the formation of small pollen grains. Two years later, Fulmer (25) reported polyspory in this species and thought that the supernumerary microspores had been produced by isolated chromosomes in the cytoplasm although he did not absolutely determine their origin.

The Onagraceae have been given much detailed study since the appearance of De Vries' mutation theory in 1900. Beer (4) notes in some Onagraceae the presence of two nuclei, one large and the other small, in some quite young pollen grains. He suggests that the dwarf nuclei are likely caused by irregularities similar to those described for *Hemerocallis fulva*.

Gates has published a series of papers on the cytological development of the pollen mother cells of *Oenothera Lamarckiana*, *O. lata*, *O. gigas*, and hybrids of these forms. In his preliminary note (27), he refers to some irregularities in *O. lata*  $\times$  *Lamarckiana*. He draws attention to the fact that like irregularities exist in *Hemerocallis fulva*, *Syringa rothomagensis* (a hybrid), *Ribes* hybrids, and hybrid pigeons, and suggests that this may be a characteristic useful in distinguishing hybrids. His paper (28) shows that when an *O. Lamarckiana* form is produced by a cross of two forms, the hybrid is triploid. In another paper appearing the same year (29), he shows that extruded chromosomes and small nuclei in the cytoplasm occur in hybrid *Oenotheras*, and he thinks that irregular tetrad-formation puts under suspicion the purity of any plant in which these phenomena are found. He points out that these forms in which polyspory has been reported by Wille (76) and others are either known hybrids or are cultivated species and quite likely have become hybridized. Gates (30) reports irregular distribution of the chromosomes in both triploid and tetraploid forms. The point I wish to emphasize, because it parallels the condition found in triploid Rubi, is the reduction of the 21 chromosomes to 10 and 11, rarely 9 and 12. In a final note, appearing in a later paper, Gates (31) attributes sterility in *Oenotheras* to unpaired chromosomes in the pollen.

Lutz (49) finds polyploidy general in hybrid and mutant *Oenotheras* and concludes that correlation exists between external appearance and chromosome number. The work of Davis (15) contrasts the meiotic divisions of *O. biennis* with those of *O. grandiflora* Ait. In the latter, the chromosomes arrange themselves much more regularly at the equator and the heterotypic division is much more regular than in the former. In *O. biennis* the chromosomes are scattered in their distribution at the equatorial plate. Davis (16) points out that the genus *Oenothera* is not a valid one for breeding experiments, for so many of the species under consideration are not genetically pure. More recent is the work of Cleland (12) on the reduction divisions of *O. franciscana* B., one of our most stable species. He finds the regular reduction of the pollen mother cell typical, parallel to that found for *O. grandiflora* Ait. and in marked contrast to that found in less stable species. He says that until we know the cause of sterility in members of the *Lamarckiana* group it is impossible to tell whether "mutants" thrown by them are indeed mutants or whether they are Mendelian segregates.

Van Overeem (75) has concentrated his cytological investigations on two *semigigas* forms of *Oenothera*. Both are triploid and the result of crosses between mutants and normal forms, called by De Vries "Halbmутanten." The reduction of the chromosomes to 10 and 11 agrees with that described by Gates (30). The distribution of the chromosomes during meiosis is irregular and frequently leads to polyspory.

Twenty-five years of study in the genus *Oenothera* has changed much our concept of a mutant. Lotsy (50) points out that the segregation found

by van Overeem (75) simulates Mendelian segregation to an astonishing degree and that this condition usually follows unequal distribution of the chromosomes.

The fundamental work of Rosenberg on a *Drosera* hybrid has become classic. His first paper (57) on *Drosera longifolia*  $\times$  *D. rotundifolia* showed that he was working on a hybrid that was the result of the union of two sex cells with different chromosome numbers, *D. longifolia* having 20 gametophytic chromosomes and *D. rotundifolia* 10. The hybrid has as its sporophytic number the sum of the gametophytic chromosomes of its parents, viz., 30. In the diakinesis are seen ten paired and ten single chromosomes—in all, 30. His next paper (58) followed more carefully the different stages in pollen-formation. He found that the bivalent and univalent chromosomes behave very differently. The latter seem to lose their attachment to the spindle fibers and are left to wander during the heterotypic division. The bivalent chromosomes behave in a perfectly regular manner, but the univalents lag in their approach to the nuclear plate and many are found on the spindle during the heterotypic anaphase. As a consequence of this lagging, many of the univalents are left in the cytoplasm instead of entering the daughter nuclei. The homoeotypic division is more regular, but quite frequently minor spindles as well as the two major spindles can be seen at the homoeotypic metaphase. These result from chromosomes extruded during the heterotypic division. The outcome of such irregularities is the formation of more than four pollen grains from one pollen mother cell.

In the *Drosera* hybrid, Rosenberg has described the division of what is now almost considered a type meiosis for a hybrid that has resulted from the union of sex cells with different chromosome numbers. A piece of cytological work very similar to this was done by Yasui (77) on artificially produced *Papaver* hybrids.

Before entering upon a discussion of the findings in *Rubus*, I shall review briefly other publications describing irregularities in meiosis in order to show the wide occurrence of irregular chromosome-distribution in heterozygous forms.

The outstanding cytological investigation of the spore condition in hybrid ferns is that of Farmer and Digby (20) on varietal and hybrid forms of *Polypodium*. The hybrid *Polypodium Schneideri*, resulting from a cross of *P. vulgare* and *P. aureum*, has a varying number of chromosomes, from 95 to 125, which suggests the sum of the numbers of the two parents, the former having 35, the latter about 90. From a study of this hybrid and of varietal forms of *P. vulgare*, they found that in spore-formation the chromosomes are frequently distributed in an irregular manner, but the hybrid shows much more frequent abnormalities in its spore-development.

In the monocotyledons, Belling (6) reports a triploid *Canna* in which the chromosomes become trivalent instead of bivalent previous to the reduction divisions. Each of these trivalent chromosomes separates on the spindle

into two pieces, one carrying two univalents and the other one. All of these move in a random manner to the poles.

Several important cytological investigations have been made of the sex-cell development of our cereals. Collins (14) has suggested that corn, *Zea Mays*, is a hybrid. Kuwada has made in two papers (45, 46) a cytological investigation of the chromosome number in various strains of *Zea*. He finds great variability in size and number of chromosomes in the different races which, he thinks, behave as if they resulted from a doubling of chromosomes of some original form which had probably 6 gametophytic chromosomes.

The cytological research on wheat has been extensive. Sakamura (64) is of the opinion that the chromosome number has increased from 7 through crossing. He finds increase in chromosome number, and the irregular distribution of the chromosomes is the cause of sterility in hybrids. Kihara (41) finds in his work on hybrid wheats that meiotic divisions are similar in all crosses and describes a cross of *Triticum polonicum*  $\times$  *T. Spelta*. The hybrid is a pentaploid form, giving at diakinesis 14 bivalent chromosomes and 7 univalents, and the pollen-formation is, in every detail, identical with that reported for *Drosera*. The embryo-sac mother cell shows the same irregularities as does the pollen mother cell. His second paper was written after a cytological study of many  $F_1$  and later generations of six different wheat crosses. In these he found varying chromosome numbers. The experimental and cytological work of Sax (65) divides the wheat species according to the gametophytic chromosome number. In inter-group crosses he obtained forms with 7 bivalent and 7 univalent chromosomes, or 14 bivalent and 7 univalent chromosomes. The 7 univalent chromosomes behave in an irregular manner during pollen-formation, but he did not report polyspory in any hybrid studied. The writer has observed very similar conditions in hybrid *Iris*es.

De Mol (53) has recently studied commercial forms of hyacinths of hybrid origin and has found that the chromosome number varies with the varying types.

The banana, a fruit that has been much cultivated, has been investigated cytologically by Tischler (73), and he describes the chromosome condition. He found 8-, 16-, and 24-chromosome forms, but he does not think it safe to interpret the irregularities until the varieties are studied in their wild habitats. The more recent work of d'Angremond (2) was an investigation of two quite stable species and two variable, sterile species. The stable, fertile species have a regular meiosis and the pollen is morphologically good. The two sterile species have irregularities connected with pollen-development, and quite frequently more than four nuclei develop from one pollen mother cell. As high as 11 grains are produced in rare cases. He is inclined to believe that the two sterile forms are hybrids and in this way accounts for the irregularities in the reduction division.

Tischler, to whom we are indebted for considerable cytological investigations of hybrids, has summed up much of the previous work on plants that show varying chromosome number. In his review of the chromosomes found in various plants (74), he places the *Bryonia* hybrid, which he had studied earlier (72), in the group of hybrids resulting from the crossing of species with unequal chromosome numbers. The hybrid, he found, is characterized by irregularities in chromosome-distribution during the heterotypic division. These involve lagging chromosomes and their extrusion into the cytoplasm, where they either deteriorate or become nuclei for dwarf pollen grains. In some cases he found nuclei which were connected by lagging chromosomes, giving a figure he called "pseudoamitosis." His work on a *Ribes* hybrid (71) followed the development of the pollen mother cell. The infrequent irregularities of this hybrid may be explained by the fact that the parents did not differ in chromosome number, for he found the number the same in the hybrid as in one of the parents.

Juel (40) published an interesting report on conditions found in *Syringa rothomagensis*, a hybrid. He found that the chromatin materials from the two parents did not seem to fuse, but kept, more or less, their individuality, and as a consequence many irregularities resulted in the pollen-formation. He states:

Aus meiner Untersuchung von der Pollenbildung bei *S. rothomagensis* geht jedenfalls hervor, dass Sterilität hier durch Abnormalitäten der Tetradentheilung hervorgerufen wird.

Several papers have appeared on the formation of pollen in *Fuchsia*. Anderson (1) made two crosses in this genus, one, *F. procumbens* × *Empress* (a hybrid), resulting in a triple hybrid. McAvoy (51) studied the pollen-development of several cultivated *Fuchsias*, which, she stated, were probably hybrids. There were irregularities in the pollen-development, and she points out that they may be directly connected with the supposed hybrid nature of these greenhouse varieties. Beer (5) studied this genus to determine the cause of the formation of supernumerary pollen grains. He found the heterotypic divisions very irregular, and the extrusion of chromosomes leads to the formation of micronuclei. In some extreme cases chromosomes fail to separate and the whole cell degenerates. During the homoeotypic division the small as well as the large nuclei undergo karyokinesis.

A cytological study of the chromosomes found in *Primula kewensis* was published by Digby (17). The parents, *P. floribunda* and *P. verticillata*, of the hybrid *P. kewensis*, both have 9 as their gametophytic chromosome number. Sterile forms of *P. kewensis* have the same chromosome number as that of the parents. An  $F_2$  from this cross was finally obtained which is characterized by a doubling of the typical parental number of chromosomes. This number is repeated in the seedling from *P. kewensis farinosa* and reappeared in the *P. kewensis* form which resulted from a cross of *P. verticillata* with *P. floribunda isobellina*. From this we see that the only hybrid that was fertile was tetraploid, the diploid hybrid being sterile.



The Compositae are generally ranked with the polymorphic families of the angiosperms. Cytological work on many different genera, as Tischler (72) points out, has shown that there are many forms that reproduce apomictically, an embryo being developed asexually. Rosenberg (60) finds that *Hieracium auricula* has 9 chromosomes as the reduced number. Three other forms studied, *H. excellens*, *H. Pilosella*, and *H. flagellare*, show an increase above the 9, and associated with this polyploid condition in the reduction phase is a failure to form bivalent chromosomes, and consequently, at diakinesis, bivalent and univalent chromosomes are present. He thinks from the behavior of crosses of the above-mentioned forms that the species are themselves possible hybrids. Hybrids resulting from the crosses of these and other variable species show universally the presence of both univalent and bivalent chromosomes. He has divided the irregularities found into three classes: the first, in which univalents and bivalents are both present, the latter causing striking irregularities in both the heterotypic and the homoeotypic division. The second type he calls "halbheterotypisch," for the chromosomes do not seem to collect at the equator and the spindle is poorly formed, or, in extreme cases, no spindle appears at all, and as a result the daughter cells have very unequal chromosome numbers. In the third type the division is typically somatic in appearance, and so no reduction in chromosome number occurs.

Farr (21) found, in Chrysanthemum, conditions very similar to those reported by Juel (39) in the development of the tetrad of *Hemerocallis fulva*. Tahara (70) has extended the research to many species and found polyploidy general, *C. arcticum* having 45 as its reduced chromosome number. It is noteworthy that the chromosome number in species of Chrysanthemum is 9 or a multiple of 9.

A study of *Crepis* by Rosenberg (61) has shown how polyploid forms have originated in this genus. He finds that occasionally a small chromosome lags, and, if this is carried over with its mate in the heterotypic anaphase to the daughter cell, one of the two will have this small chromosome duplicated. If the cell having an additional chromosome fertilizes a like egg, the resulting form will have two small chromosomes instead of one; and in *C. tectorum* this is the condition found, while in *C. rubra* there are three small chromosomes. Babcock and Collins (3) studied a hybrid, *C. capillaris*  $\times$  *C. tectorum*, and, although the plant did not mature, they were able to show that the new plant had as its chromosome number the sum of the haploid numbers of the parents.

Holmgren (36) finds in the pollen-development of *Eupatorium glandulosum* three types quite parallel to those described by Rosenberg (60). In some figures the heterotypic division is normal, in others it is very irregular, in consequence of the presence of nonpairing chromosomes, and in still others no chromosomes pair but the division is typical in character. He says that "normale Tetraden sind in dem untersuchten Material bisher noch nicht gefunden worden."

Ishikawa (37) has shown in a tabular way variations found in the chromosome numbers of different genera of the Compositae, the result of his work on *Dahlia* being included.

#### CONCLUSION

The pioneer study of the chromosome numbers in the pollen mother cells of roses was that of Rosenberg (59) on *Rosa canina* var. *persaticifolia* A. & M. and *R. glauca Afzeliana* Fr. var. *dilatans* At. The recent studies of the genus *Rosa* by Blackburn and Harrison (8) and by Täckholm (69) have made it clear that a study of the chromosome number is essential in determining the relations of variable hybrid species.

A study of the chromosome number and behavior in *Rubus* has brought out many striking parallels to the conditions found by these European authors in *Rosa*.

The diploid species of *Rubus* show a regular pairing and distribution of chromosomes at meiosis. These phenomena seem a reasonably certain test of homozygous species. The species *R. neglectus* is undoubtedly an exception, for it is unquestionably a hybrid and seems to agree closely with the diploid rose hybrids described by Täckholm. This exception forces me to conclude that degeneration of the pollen grain is not always associated with irregular mitosis, etc. The species *R. alleghaniensis* is apparently a very stable species. The remaining diploid Rubi of the Eubatus group I rank with this much-studied form on account of the similarity in the process of pollen-formation, which normally leads to morphologically good pollen. A fact that may be of significance is the self-sterility of *R. alleghaniensis*, as shown by Peitersen (55), in contrast to the other blackberries he studied, which either are self-fertile or form seeds asexually.

Tetraploid Rubi were found to be quite regular in their pollen-formation. Chromosome-duplication has been the outcome of hybridization in many genera, and, though the cytological study reveals no other characters that can be associated with hybridization, I class such Rubi as probable hybrids. The significance of these tetraploid species is the rôle they play in crossing with diploid species.

Many of our New England Rubi are triploid. The chromosomes in these come to pairing very slowly, and a typical diakinesis is found extremely rarely. The reduction to 10 chromosomes may seem a little out of the usual procedure, but Belling (6) finds that triploid *Canna* and *Datura* form trivalent instead of bivalent chromosomes at the heterotypic prophase. I find that one chromosome seems to be trivalent. The more usual condition described for triploid species is that outlined by Rosenberg for *Drosera*. The fate of 7 of the 21 chromosomes seems very variable, in contrast to the regular distribution of the remaining 14. Both the heterotypic and the homoeotypic divisions seem typical of primary hybrids.

The pentaploid, hexaploid, and octoploid species show all the characteristics found in the triploid species. One, *R. plicatifolius*, shows polypspory, a condition that is the outcome of the irregular distribution of chromosomes. Polypspory is not always reached in *Rubus*. I think that it occurs in many species, but is passed unnoticed. Its presence can generally be predicted by the number of chromosomes extruded during the heterotypic division.

This cytological study of pollen-formation in the *Eubatus* group of *Rubus* has been an aid in drawing a clear line of distinction between heterozygous and homozygous forms. The hybrid character in a species is brought out in the general behavior of the reduction divisions. It may reasonably be inferred from the above-described conditions that the blackberries have increased in the wild through natural hybridization.

It is not easy to say at what time this hybridization has taken place. Did it occur in pre-glacial times? Focke supports this view, and considers that *R. Hochstetterorum* Seub., of the Azores; *R. grandifolius* Lowe, of Madeira; and *R. numidicus*, of north Africa, in consequence of their discrete distribution, have outlived the parents that were obliterated during the ice age. This view, very apparently, presents serious difficulties, and unless many of our blackberries can be shown to reproduce asexually, as described by Täckholm and others for *Canina* roses, the evidence favors the view that hybrid *Rubi* are being produced in the wild at the present time. This opinion is supported by the fact that, in nature, hybrids generally exist in the near vicinity of the two supposed parents, and secondly by the fact that experimental plant breeders have produced hybrids identical with those found in nature. Until a cytological study is made of the macrospore mother cells of these hybrids it cannot be definitely stated whether sexual fusion has or has not taken place, and, if it has, the question arises, how many chromosomes does the egg contribute to the offspring? Not until such research has been done can one calculate the number of chromosomes the pollen contributes to the egg in order to produce an offspring with the same chromosome number as the parent. Both Lidforss (47) and Peiterzen (55) have shown that heterozygous *Rubi* do not exhibit true segregation, but act as if many factors were involved.

In a brief paragraph I wish to call attention to a short summary of the work done in this laboratory: "Polyploidy, Polypspory, and Hybridism in the Angiosperms," appearing in *Science*, May 12, 1922. Professor Jeffrey's study of this subject has extended over many years. The writer gives in this paper the results of his study of the genus *Rubus*, while the second junior author, Mr. Penland, has prepared for publication his study of the genus *Rosa*, to appear in the *Botanical Gazette*.

## SUMMARY

Rubus species can be divided into two major classes: (1) Diploid species, in which I find the gametophytic chromosome number to be 7, the sporophytic 14. (2) Polyploid species, including triploid, tetraploid, pentaploid, hexaploid, and octoploid.

The diploid species are characterized by a regular distribution of chromosomes and regular pollen-formation. This, I consider, is a pretty reliable criterion of a pure species.

The polyploid species are characterized, not only by an increase of chromosomes above the diploid number, but by striking irregularities in chromosome distribution and irregular pollen-formation, leading frequently to polycary and polyspory. In view of the similarity of these irregularities to those found in known hybrids, I consider that this group is made up of hybrid species and forms.

This cytological study, substantiated by morphological and genetical studies, has shown the hybrid nature of a large number of species in this polymorphic genus of the Rosaceae.

These heterozygous forms show the following characters: pollen sterility, variability of offspring, polyploidy, irregular chromosome distribution, polycary, and polyspory—all features clearly associated with hybrid forms.

It seems, therefore, that in this genus multiplication of species has taken place by hybridization in their natural habitats. Some hybrids may date back to pre-glacial times, but there is much evidence to show that many of the so-called species are able to originate at the present time wherever inter-fertile species are present.

In a closing paragraph, I wish to express my sincere thanks to Prof. C. S. Sargent of the Arnold Arboretum for the privilege of collecting the material necessary for this work. I desire, also, to express my obligation to Miss Day, librarian of the Gray Herbarium, for assistance in securing literature, and to others who have kindly aided me in carrying out this study. This work has been carried on in the laboratories of plant morphology of Harvard University under the direction of Prof. E. C. Jeffrey, and to him I am much indebted for suggestions and assistance.

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### DESCRIPTION OF PLATES

The plates and text figures were kept in their true proportions by drawing at the height of the microscopic stage with a camera lucida. I used a 1.5-mm. Zeiss apochromatic objective and a no. 12 compensating ocular, and the drawings were reduced one third in photographing.

### PLATE XI

#### *Rubus occidentalis* L. Pollen-formation.

- FIG. 1. Early heterotypic prophase, showing 14 univalent chromosomes.
- FIG. 2. Diakinesis, showing 7 bivalent chromosomes.
- FIG. 3. Late heterotypic metaphase.
- FIG. 4. Heterotypic anaphase.
- FIG. 5. Late heterotypic anaphase.
- FIG. 6. Interkinesis.
- FIG. 7. Homoeotypic metaphase.
- FIG. 8. Homoeotypic anaphase.
- FIG. 9. Late homeotypic telophase.
- FIG. 10. Tetrad.



## PLATE XII

*Rubus caesius* var. *turkestanicus* Regel. Pollen-formation.

- FIG. 1. Early heterotypic prophase, just previous to pairing of the univalent chromosomes.  
FIG. 2. Prophase, two chromosomes still unpaired.  
FIG. 3. Diakinesis, showing 14 bivalent chromosomes.  
FIGS. 4, 5. Heterotypic anaphases.  
FIG. 6. Late heterotypic telophase.  
FIG. 7. Interkinesis.  
FIG. 8. Homoeotypic metaphase.  
FIG. 9. Homoeotypic anaphase.  
FIG. 10. Late homoeotypic telophase.  
FIG. 11. Four daughter nuclei just previous to cell-plate formation.  
FIG. 12. Tetrad.

## PLATE XIII

*Rubus glandicaulis* Blanchard. Pollen-formation.

- FIG. 1. Prophase of a cell in the developing ovule wall, showing 21 sporophytic chromosomes.  
FIG. 2. Heterotypic prophase, showing 21 univalent chromosomes.  
FIG. 3. Diakinesis, showing 10 chromosomes.  
FIG. 4. Heterotypic metaphase.  
FIG. 5. Early heterotypic anaphase.  
FIG. 6. Late heterotypic anaphase, showing 7 chromosomes lagging on the spindle.  
FIG. 7. Interkinesis.  
FIG. 8. Homoeotypic metaphase.  
FIG. 9. Homoeotypic anaphase.  
FIG. 10. Interkinesis, showing 2 chromosomes in the cytoplasm.  
FIG. 11. Homoeotypic metaphase, with a third dwarf spindle to the left in the cell.  
FIG. 12. Tetrad.

## PLATE XIV

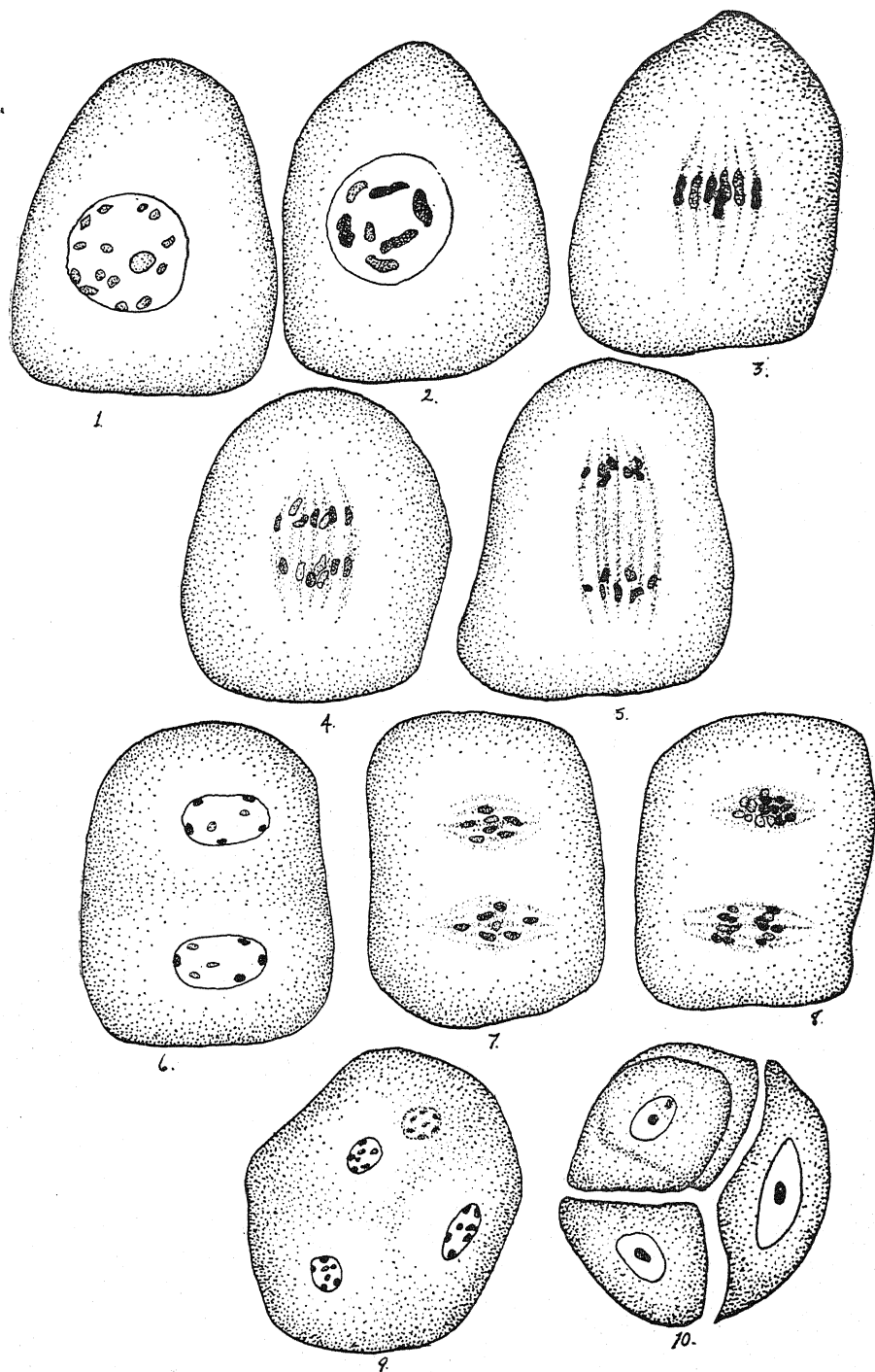
*Rubus plicatifolius* Blanchard. Pollen-formation.

- FIG. 1. Pollen mother cell, just previous to meiosis.  
FIG. 2. Heterotypic prophase, where practically all chromosomes are bivalent.  
FIG. 3. Heterotypic metaphase.  
FIG. 4. Early heterotypic anaphase.  
FIG. 5. Late heterotypic anaphase.  
FIG. 6. Interkinesis, showing extruded chromosomes in the cytoplasm.  
FIG. 7. Homoeotypic metaphase, showing chromosomes in the cytoplasm to the left of the cell.  
FIG. 8. Late homoeotypic metaphase; a group of extruded chromosomes show preparation for division.  
FIG. 9. Homoeotypic anaphase.  
FIG. 10. Late homoeotypic anaphase.  
FIG. 11. Late homoeotypic telophase, showing 4 major nuclei, 2 dwarf nuclei, and 2 chromosomes in the cytoplasm.  
FIG. 12. Tetrad, but dwarf pollen grain present also.

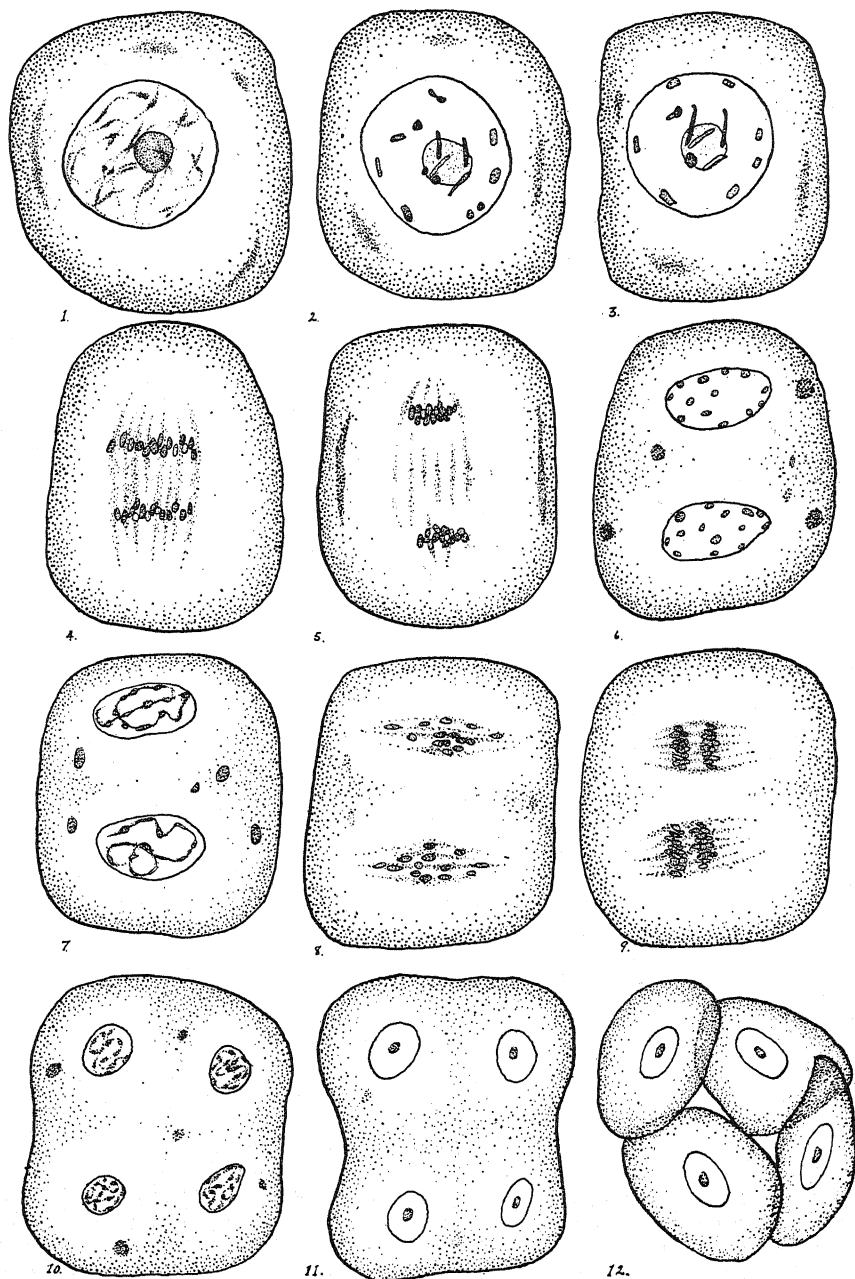
## PLATE XV

An octoploid *Rubus*. Pollen-formation.

- FIG. 1. Diakinesis.  
FIG. 2. Heterotypic metaphase.  
FIGS. 3, 4. Heterotypic anaphase, early and late.  
FIG. 5. Interkinesis, showing chromosomes in the cytoplasm.  
FIG. 6. Homoeotypic metaphase.  
FIG. 7. Late homoeotypic metaphase.  
FIG. 8. Early homoeotypic anaphase.  
FIG. 9. Late homoeotypic anaphase.  
FIG. 10. Late homoeotypic telophase.  
FIG. 11. Mother cell with 4 nuclei and chromatin masses in the cytoplasm from previously extruded chromosomes.  
FIG. 12. Tetrad.
- LABORATORY OF PLANT MORPHOLOGY,  
HARVARD UNIVERSITY

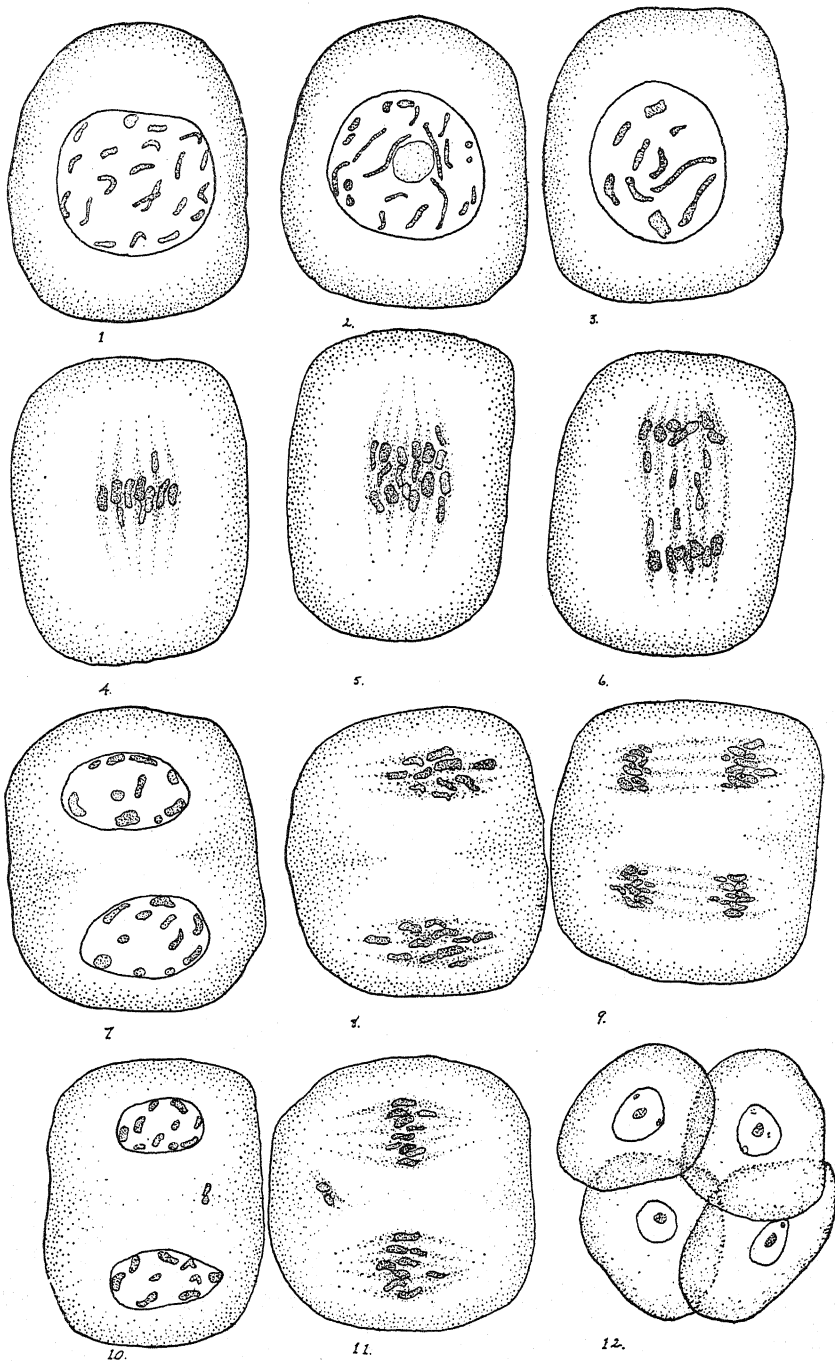






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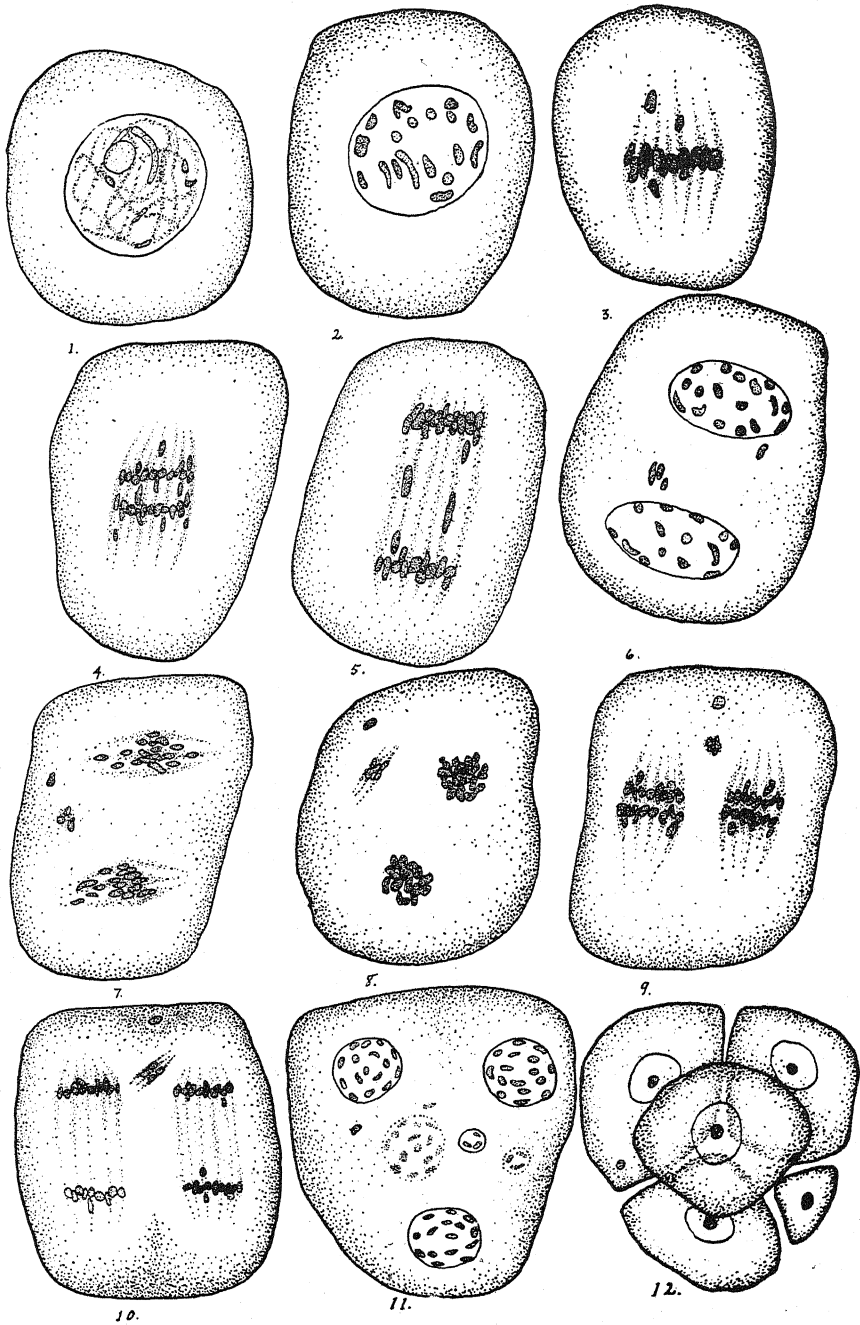




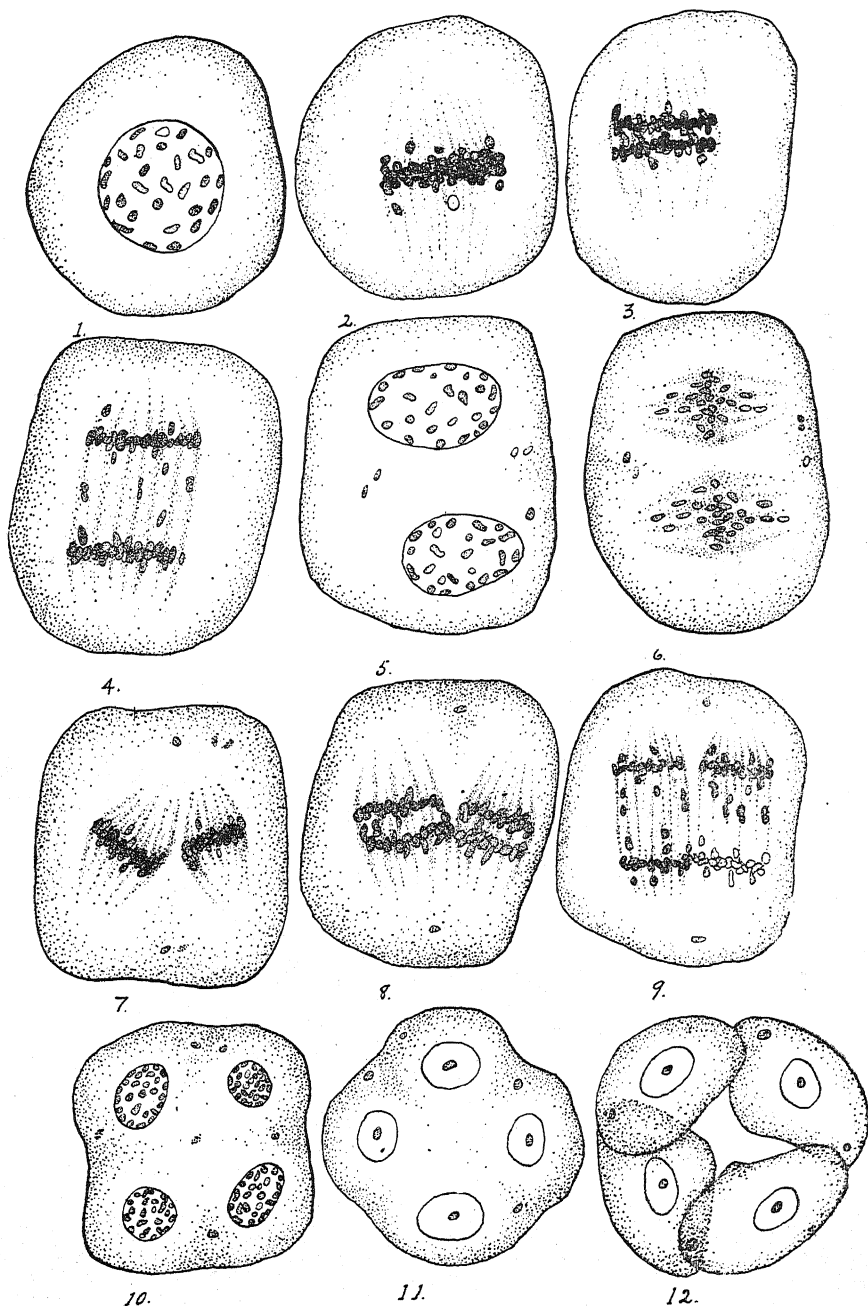
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## THE PHYSIOLOGY OF POLLEN II. FURTHER CONSIDERATIONS REGARDING THE REQUIREMENTS FOR GROWTH<sup>1</sup>

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(Received for publication July 5, 1923)

### NITROGEN-ASSIMILATION AND THE POSSIBLE RÔLE OF GROWTH- ACCESSORY FACTORS

Tokugawa (1914)<sup>2</sup> noted that the addition of some protein materials to pollen cultures often more than doubled the growth. Knight (1917), coöperating with Miss Eckerson, reports that, with the germination and growth of the pollen tubes of apple, asparagin makes its appearance. Miss Eckerson found, moreover, that the addition of traces of asparagin to artificial cultures of apple pollen accelerates the rate and increases the total amount of pollen-tube growth.

The writer has made numerous tests of the effect of the acid amide asparagin on pollen-tube growth without, however, observing any case in which its addition to the cane-sugar medium increased growth. Pollen of *Nicotiana*, *Cucumis*, *Scilla*, *Vinca*, and several species of *Pyrus* was tried on cultures in which the concentration of asparagin ranged from 1/2000 to 1 percent. At the higher concentrations growth was markedly retarded. The amino acid glycocoll similarly failed to give positive results. Some evidence was secured, however, to show that the addition of traces of peptone promotes growth. Inorganic forms of nitrogen, as  $\text{KNO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$ , gave negative results. Growth was checked by these salts even when present in extremely small amounts.

A very considerable improvement in the artificial cultures was secured, however, through the incorporation of sterile yeast. This was prepared by working up a cake of Fleischmann's compressed yeast with 100 cc. of water and boiling the suspension 1 to 2 minutes. Because of the tapioca flour in the commercial article, the resulting preparation is thick and viscid. If

<sup>1</sup> Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

<sup>2</sup> The references are to literature cited at the end of the fourth paper of this series, to appear in a future issue of this JOURNAL.

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the flour is separated by sedimentation before boiling, a clear yellowish extract of the yeast can be prepared which still retains the growth-promoting properties of the cruder material. This latter, however, is more readily prepared and was used in subsequent tests.

TABLE 1. *The Effect on the Growth of Pollen Tubes of Cucumis sativus of Adding Live Yeast, Sterile Compressed Yeast, and Sterile Brewer's Yeast to Sugar-agar Culture Medium (Lengths Given in Microns)*

Test	Check		Sterile Compressed Yeast		Sterile Brewer's Yeast		Live Yeast	
	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length
1.....	99	422	100	571	81	420	—	—
2.....	58	600	63	626	69	579	65	324
Average....	157	488	163	592	150	494	65	324

A sample of brewer's yeast was found unsatisfactory because of impurities. The material obtained was taken from the bottom of a vat and contained a considerable amount of sediment difficult to remove. Living yeast, added to the sugar medium, gave rise to alcoholic fermentation which checked growth. The results of comparative tests between compressed yeast and the sample of brewer's yeast mentioned above, both prepared by boiling, and living yeast are given in table 1.

TABLE 2. *Influence on the Growth of Pollen Tubes of the Addition of Sterile Yeast to Culture Media (Lengths Given in Microns)*

Test No.	Test Species	Concentration Sugar (percent)	Without Yeast		With Sterile Yeast, 2 drops per 25 cc.		Percent Increase
			No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	
1. <i>Cucumis sativus</i> .....		10	111	573	91	1270	121
2. <i>Cucumis sativus</i> .....		10	43	753	71	932	24
3. <i>Cucumis sativus</i> .....		10	74	1710	78	758	-55
4. <i>Cucumis sativus</i> .....		10	69	261	59	707	170
5. <i>Primula obconica</i> (long-styled) ..		10	64	175	101	310	77
6. <i>Primula obconica</i> (long-styled) ..		10	106	171	109	341	101
7. <i>Primula obconica</i> (short-styled) ..		10	96	326	100	496	53
8. <i>Primula obconica</i> (short-styled) ..		10	107	365	114	551	51
9. <i>Primula obconica</i> (long-styled) ..		10	86	67	114	124	85
10. <i>Primula obconica</i> (short-styled) ..		10	111	133	111	218	65
11. <i>Lythrum salicaria</i> (green pollen) ..		15	26	379	40	1503	296
12. <i>Lythrum salicaria</i> (green pollen) ..		15	34	671	40	863	28
13. <i>Lythrum salicaria</i> (green pollen) ..		15	69	590	89	1279	117
14. <i>Lythrum salicaria</i> (yellow pollen) ..		15	48	277	53	1120	304
15. <i>Lythrum salicaria</i> (yellow pollen) ..		15	47	473	58	532	13
16. <i>Lythrum salicaria</i> (yellow pollen) ..		15	60	439	55	840	92

Table 2 gives the results obtained in 16 tests with pollen of three different species. The boiled yeast was added to the appropriate cultures at the rate



of two drops per 25 cc. of the sugar-agar medium. When pollen of *Cucumis* and *Primula* was used, germination was almost perfect throughout. With *Lythrum*, on the other hand, the percentage of grains germinating was lower and variable, though independent apparently of the yeast in the medium, as table 3 shows.

TABLE 3. *Percentage Germination of Pollen of Lythrum salicaria on Media with and without Sterile Yeast*

Test	Percent Germination	
	With Yeast	Without Yeast
11.....	48.7	37.7
12.....	54.8	53.1
13.....	89.9	85.2
14.....	75.7	49.0
15.....	79.4	76.3
16.....	57.9	69.8

The increases in growth on the sterile yeast media are for the most part very considerable. In test 3 alone, in which cucumber pollen was used, the plain sugar culture gave a greater average growth. In the other three tests in which pollen from the same form was used, the increases with the addition of yeast were 24, 121, and 170 percent respectively. It was early found that with *Cucumis* extreme care was necessary to get uniform samples of pollen. The results of trials made on a constant medium justified the precaution that was taken in the experiments of securing material for a given test not only from one flower but from a single anther within the flower. Of the causes contributing to the variability in the behavior of pollen from different anthers of the same flower, nothing is known. Differences in exposure to light may be responsible, although we have no evidence to show that such is the case.

During the course of experiments with other kinds of pollen, the results of which are not included in table 2, it was quite obvious that when the yeast preparation was added growth was materially greater than on media lacking it.

There is an increasing amount of evidence that the addition of extracts of fresh plant tissues of various sorts stimulates the growth of pollen tubes on artificial cultures. East and Park (1918) obtained some evidence to show that stigmatic extracts of *Nicotiana angustifolia* and *N. forgetiana* promoted the growth of pollen tubes of *Nicotiana in vitro*. Knowlton (1922) observed that the percentage of germination of *Antirrhinum* pollen was greatly increased by the addition of minute amounts of crushed stigma to cultures of the same species. Pollen of this form which had been stored in oxygen at 10°-22° C. for 670 days failed to grow in sugar solutions, but, on the addition of a piece of stigma, 10 percent germination was secured. Pollen kept in air for the same length of time, similarly would not germinate in sugar solutions, but gave 50 percent germination on the addition of stigmatic tissue. Maize pollen, which can be germinated *in vitro* only with difficulty,

Knowlton found did not respond to the addition of stigmatic parts or decoctions of the stigma. Tokugawa (1914) obtained similar results when crushed stigmatic tissues were incorporated in pollen cultures of a number of species not readily grown *in vitro*. It is quite probable that in these cases the media were unadjusted in some other important respect for the growth of the particular species used. The important point is that, with forms which do lend themselves to present artificial culture methods, growth is appreciably enhanced by the addition of some fresh plant tissues.

While the present writer's work on this point is not extensive, some further interesting facts have been secured. It has been observed that the crushed stigmas of *Nicotiana* added to agar cultures containing 15 percent cane sugar regularly increase the growth, not only of the pollen tubes of *Nicotiana*, but also of those of other forms growing at this concentration of sugar. Crushed ovarian tissue gave similar results. Striking increases in growth were secured on the addition of 1 drop of extract of raw potato to 25 cc. of sugar-agar medium. The average length of the pollen tubes of *Cucumis* was thus increased 43 percent. Larger amounts of potato extract tend to check growth. Tomato extract, which is very acid, retarded growth except in high dilution.

To what must we attribute the growth-promoting power of these sterile yeast and fresh plant materials? The evidence at hand does not permit a satisfactory answer. The fact that such preparations are complex leaves us in the dark as to the chemical nature of the active substances, and even precludes at this stage a definite conclusion that the gynoeceum and potato extracts and the yeast preparation fall in the same category as regards their growth-promoting properties.

Some further evidence is available, however, on the nature of the material contributed by the yeast. In table 4 the results of experiments to determine the heat stability of its growth-promoting substances are summarized.

TABLE 4. *Influence on the Growth of Pollen of Cucumis sativus of Adding to the Culture Medium Yeast Boiled 1 Minute, 15 Minutes, and Autoclaved for 45 Minutes at 120°C. (Measurements Given in Microns)*

Test	Check without Yeast		With Yeast Boiled 1 Minute		With Yeast Boiled 15 Minutes		With Yeast Autoclaved for 45 Min. at 120° C.	
	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length
1.....	167	494	172	571	157	608	—	—
2.....	97	647	129	1369	—	—	144	1255
3.....	109	463	114	661	—	—	115	534
4.....	123	506	110	702	—	—	115	612

In these tests *Cucumis* pollen was used. A check culture consisting of 2 percent agar and 10 percent cane sugar was made for comparison. In

the other cultures, yeast prepared as indicated in the table was added at the rate of 2 drops per 25 cc. of medium. Boiling for 15 minutes does not result in any diminution of the growth-promoting power of the yeast. Autoclaving for 45 minutes at 120° C. diminishes its potency somewhat, but the material still retains its original properties to a marked degree.

It would not be surprising to find that the expressed juice of the other plant tissues mentioned reacted similarly to heat. The high peroxidase content of the potato may enhance its value somewhat, but the similarity of the growth-promoting powers of all the materials mentioned above suggests that some constituent common to all is responsible. It is not improbable, though, that the growing tubes utilize more than a single constituent of the sterile yeast. Whatever these substances may be, they are effective in small amounts. Tests showed that the yeast, as would be expected, loses its power to invert cane sugar in the boiling. Presumably all the enzymes present in the living cell are thus destroyed. Since an excess of carbohydrates in the form of cane sugar was already present in the culture, it is unlikely that the yeast contributes further in this regard. As will be seen later, the mineral constituents may account for a small part of the increased growth obtained. What of the remainder? Perhaps some water-soluble, heat-stable nitrogenous compound, a protein derivative, is responsible. Or possibly the growth-accessory substances, whatever their chemical nature may be, that Thjötta and Avery (1921) have found in plant tissues promoting the growth of hemophilic bacilli are the active substances. Dutcher (1918) has demonstrated the presence of the vitamin water-soluble B in maize pollen. Nothing is known, however, of the significance of such substances in pollen-tube metabolism.

#### OSMOTIC RELATIONS

A source of no little annoyance and perplexity to those who have attempted to cultivate pollen artificially has been the bursting of pollen and pollen tubes. No satisfactory explanation of this phenomenon has as yet been brought forward. It has been noted repeatedly that the amount of bursting bears no clear-cut relation to the concentration of sugar in the culture. Van Tieghem (1869), Correns (1889), Molisch (1893), and Lidforss (1896) independently have concluded from this fact that the amount of bursting is not related to the osmotic force of the surrounding medium. With this conclusion we can not agree. While additional factors associated with the colloidal nature of the protoplasm may perhaps be involved, the evidence favors the view that it is largely an osmotic phenomenon. The conclusion that it is not an unbalanced osmotic condition that is the cause of bursting has been based on the assumption that the protoplasmic surface of the pollen grain acts as a semipermeable membrane to sugar solutions. There is no good evidence to show that this, in general, is the case. Since, from the standpoint of culture methods, the matter is of prime importance, and

is moreover of considerable theoretical interest, we will review the evidence relating to it in some detail.

That pollen tubes of many species will grow readily on sugar solutions varying widely in concentration is attested by the results of numerous investigations. Molisch (1893) pointed out that the optimum concentration of sugar for germination varies widely. *Lilium Martagon* germinated in solutions containing from 1 to 50 percent sugar, and *Deutzia scabra* in 1- to 40-percent solutions. Pollen of some other species germinated over a much narrower range. Molisch also calls attention to the fact that the behavior of those species which germinate at high concentrations finds a parallel in the results given by certain fungi. Tokugawa (1914) states that the pollen grains of each species possess a definite turgor, and that for growth it is necessary that conditions be accommodated to this. But Tokugawa's data will not support the view that the range of sugar concentrations over which pollen grains will germinate is narrow, or that in many cases at least there is even a well defined optimum. He found, for example, that pollen of *Licorice aurea* grew in cane-sugar solutions ranging from 0.1 M to 1.8 M, and that there was little difference in the results between the concentrations 0.6 M and 1.0 M. Similar results were secured with other species. Adams (1916) secured germination of apple pollen on media containing from 5 to 30 percent cane sugar. Nicotiana pollen, as East and Park (1918) have shown, will grow on agar media containing from 0 to 30 percent sugar. Tischler (1917) found that pollen of *Plantago media* gave good germination in sugar solutions up to 50 percent. Knowlton (1922) observes that *Antirrhinum* pollen germinates best at one time in one concentration of sugar and at another time at another concentration. Our results with a number of other species are in substantial agreement with this finding. It has been our experience also that often pollen from the same anther will give quite similar results when grown on media containing very different amounts of cane sugar. In table 5 are given the results of a typical experiment with *Cucumis* pollen.

TABLE 5. *Results of an Experiment to Determine the Effect of Concentration of Cane Sugar on Total Amount of Growth of Cucumis Pollen Tubes (Measurements Given in Microns)*

	Conc. Sugar (percent)			
	10	15	20	25
No. of tubes measured.....	93	92	132	115
Average length.....	147	199	214	179

The very considerable body of evidence, showing that pollen grains will germinate and produce strong tubes in sugar solutions of widely different concentrations, suggests the conclusion that the cell membranes of the

pollen grain and its tube are or become permeable to cane sugar, and that the final result as far as osmotic pressure is concerned is the same as though the surrounding medium were water. How else may we interpret the facts? Perhaps osmotically active substances are produced within the cell, bringing it into equilibrium with the particular concentrations of sugar without. If this were so, we should expect a well defined optimum concentration of sugar for early growth, above which the pollen would plasmolyze and below which, if the wall could not sustain the pressure, the tubes would burst. But we find that *Cucumis* pollen for example will germinate almost *immediately* in each case when placed on media in which the concentration of sugar varies from 5 to 20 percent. Even at 25 percent there is no clear evidence of plasmolysis, and, while germination is somewhat slower, sufficient time does not elapse before the tube appears to encourage the view that in the interval much material of osmotic value could have been produced within the cell. We know of no mechanism inside the cell capable of such wide and rapid adjustment of sap-concentration as such an explanation would demand.

The fact that starch sometimes accumulates in pollen growing in sugar solutions is unmistakable evidence that sugar passes in, although little knowledge of the rate of diffusion is afforded thereby. Mangin (1886) noted that pollen tubes of some plants increase the size of their starch grains on sugar media. Mangin's observation has been confirmed by Tischler (1917), who, further, quotes Dodel (1878) as having found that *Pinus* pollen in concentrated sugar solutions formed many large amyllum grains. Strasburger (1872) found much greater amounts of starch in the pollen tubes of *Pinus sylvestris* growing in styler tissue than the contents of the original pollen grain could account for.

While we have found such experiments rather unsatisfactory, it does appear that, when pollen tubes are transferred from a culture of lower sugar concentration to one much higher, very little or no plasmolysis occurs. Some tubes of *Vinca minor*, grown in a 10-percent sugar culture with agar and transferred with a needle to a 50-percent solution of sugar, plasmolyzed throughout the greater part of their length only to regain their normal condition in a very few minutes. Such recovery is taken as evidence of the penetration of the sugar. Other tubes in the same transfer showed no plasmolysis. It would appear that in such cases the sugar penetrates so rapidly that no water is withdrawn from the tubes and shrinkage does not result.

Lloyd (1918) is inclined to regard bursting as due entirely to imbibition by the protoplast beyond the strength of the wall to confine it. This explanation leaves out of consideration the osmotic action of the dissolved materials in the tube and is vitiated, it seems to us, by certain facts regarding the amount of bursting that takes place when tubes grown on sugar solutions of different strengths are transferred to pure water. Lidforss (1896) placed pollen of *Lobelia cardinalis* for one hour on culture media containing 3,

2, and 1 percent of sugar respectively. On transferring the grains to distilled water, a large number of the first lot burst and a considerable number of the second; of those grown in 1 percent sugar, none burst. On Lloyd's hypothesis we are at a loss to explain these facts. On the other hand, if the pollen is somewhat permeable to cane sugar, so that, during the hour on the culture media, the original osmotic pressure of the solution within the tubes becomes increased by an amount approaching that of the surrounding solution, the tubes, when transferred to distilled water, will tend to burst the more freely the higher the concentration of the solution in which they were grown.

The evidence which Lidforss (1896) and other investigators have brought forward, showing that pollen of many species will germinate well in distilled water, indicates that the osmotic pressure developed in these cells is relatively low. Their turgor is largely due perhaps to electrolytes dissolved in the cell sap, to which the plasma membrane is less permeable than to sugars. While Martin (1913) found that the pollen grains of *Trifolium pratense* are permeable to saturated solutions of  $\text{KNO}_3$  and  $\text{NaCl}$ , we should not attach much significance to such facts, since, as the researches of Osterhout (1922) and others have shown, such materials in unbalanced solutions may cause marked changes in permeability through injury. Initially, the surface of the pollen grain may in some forms act as a semipermeable membrane to sugar solutions, for, as Anthony and Harlan (1920) found, barley pollen is plasmolyzed in high concentrations and in low ones it bursts.

The writer has found that, while bursting of *pollen grains* of *Cucumis* is little in evidence on agar media containing various concentrations of sugar, the *tubes* burst freely during their rapid elongation; or they may remain intact up to one hour after their maximum length has been reached. The simplest explanation of these facts is that pollen is fairly permeable to cane sugar and that the pollen tubes may become more so during their growth. As the sugar diffuses in, to the osmotic force of the original cell sap is added that of the solution without, and bursting results whenever the pressure exceeds the resistance of the pollen-tube wall. The permeability of the protoplasmic membrane in various kinds of pollen may be different, and in a given case may be dependent to a certain degree upon growth conditions.

#### THE EFFECTS OF SALTS, AND SOME REMARKS ON ACIDITY

It has been found again and again that the addition of electrolytes to pollen cultures hinders growth or inhibits it entirely. Lidforss (1896) observed that  $\text{NaCl}$ ,  $\text{KNO}_3$ , and  $\text{Ca}(\text{NO}_3)_2$ , even in small amounts, are toxic. Different species, however, reacted somewhat differently to these salts.  $\text{Ca}(\text{NO}_3)_2$  was very poisonous for *Nicotiana* but relatively harmless to *Lobelia* pollen.  $\text{KNO}_3$ , on the other hand, injured *Nicotiana* pollen less than that of *Lobelia*. In a further paper, Lidforss (1899 a) reports that, while fresh albumin was toxic, pollen tubes reacted chemotropically to it

after dialysis. Tokugawa (1914) found  $\text{KNO}_3$ ,  $\text{K}_3\text{PO}_4$ ,  $\text{CaSO}_4$ ,  $\text{FeSO}_4$ , and  $\text{ZnSO}_4$  injurious in various degrees to pollen from a number of different species.

The writer has observed that the addition to sugar-agar cultures of even small amounts of  $\text{NaNO}_3$ ,  $\text{NaCl}$ ,  $\text{KNO}_3$ ,  $\text{CaSO}_4$ ,  $\text{CaCl}_2$ ,  $\text{KH}_2\text{PO}_4$ , and  $\text{Na}_2\text{HPO}_4$  reduces the growth to a marked extent. The salts present in the boiled yeast preparation used in small amounts in our culture media do not retard growth, however. To test this point, a known quantity of yeast was incinerated over an open flame to a gray ash. This was taken up with water, and the salts were brought to the same concentration as in the sterile yeast preparation used. Cultures were prepared containing 1 and 2 drops of this solution per 25 cc., the latter being the concentration in which the whole yeast was being used, and the amount of growth of *Cucumis* pollen was determined on each of these media and on a check. The results are given in table 6.

TABLE 6. *The Effect of Yeast Ash on the Growth of Cucumis Pollen Tubes (Measurements Given in Microns)*

Test	Check		1 Drop Yeast-ash Solution		2 Drops Yeast-ash Solution	
	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length	No. of Tubes Measured	Average Length
1...	94	292	98	306	97	363
2...	84	365	73	310	80	483

We may reasonably assume that any toxic properties of the inorganic constituents of yeast would be manifested in greater degree when these exist as free salts than when they are partially combined with the protein complex, as is probably the case in the boiled yeast used. Our experiments indicate that in the free state yeast salts may promote pollen-tube growth to a slight extent; at least at the concentrations used they do not retard it.

Some experiments were made to determine the effect of sea water on pollen-tube growth. A series of cultures were prepared containing 2 percent agar and 10 percent cane sugar with a little sterile yeast, in which the distilled water ordinarily used was replaced with sea water to the extent of 6, 12, and 25 percent respectively. In table 7 the percentage of germina-

TABLE 7. *The Effect of Sea Water on Germination and Growth of Cucumis Pollen (Lengths Given in Microns)*

	Conc. of Sea Water			
	Check	6 percent	12 percent	25 percent
Percentage germination.....	97.7	98.1	70.0	3.8
Average length of tubes.....	561	549	284	112



tion and the amounts of growth of *Cucumis* pollen on these media and on a check without any sea water are given.

According to Dittmar's data as recalculated by Henderson (1913), the amounts of sea water here used represent concentrations of marine salts of 0.20, 0.41, and 0.85 percent. Less than 0.5 percent of sea salts depresses germination and growth markedly. In the 0.85 percent culture, but 2 grains in a total of 52 germinated, and these produced only short tubes. The injurious effects of sea water are very striking.

Interesting differences in the amount of pollen-tube growth were also found when the shred agar ordinarily used in the media was replaced by a specially prepared product, "Bactoagar." Analyses by Fellers (1916) show that this latter is somewhat lower in content of moisture, nitrogen, crude fiber, and ash than the common shred agar. Smaller but probably significant differences resulted, too, when chemically pure sucrose was used instead of the granulated cane sugar of the trade. In the experiments with these materials, the four possible combinations of agar and sugar were made and the percentage of germination and the amount of growth of *Cucumis* pollen were determined on each medium. The results are summarized in table 8, the average length of the tubes on the shred agar-commercial cane sugar medium in each test being taken as 100.

TABLE 8. *Summary of Results on the Effect of "Bactoagar" and C. P. Cane Sugar on Growth of Cucumis Pollen*

Culture Medium	Percentage of Germination (Average of 2 Tests)	Amount of Growth	
		Test 1	Test 2
Shred agar-com. sugar.....	87.5	100.0	100.0
Shred agar-c.p. sugar.....	70.9	84.8	86.1
Bactoagar-com. sugar.....	54.3	13.1	18.0
Bactoagar-c.p. sugar.....	64.1	13.7	23.8

In these two tests, 750 pollen tubes were measured. Since the respective values obtained in each experiment are of the same order, we are justified in attaching some significance to the results.

As a difference in pH was found between the two brands of agar used in the foregoing experiment, we should, before proceeding to an analysis of the results, consider the evidence, meager as it is, regarding acidity and alkalinity in relation to pollen-tube growth. Molisch (1893) observed that the stigmatic secretions of many plants are acid, turning blue litmus a deep red. He found, moreover, that pollen of *Azalea indica*, *Rhododendron ponticum*, and *R. arboreum*, which otherwise would germinate only on the pistils, grew beautifully in distilled water containing 0.01 percent malic acid or a little calcium malate. Citric acid failed to promote germination. Jost (1907) secured pollen tubes of *Rhododendron* sp. 12 mm. long

on a medium containing 5 percent cane sugar and 0.01 percent citric acid, and noted further that the pollen was very sensitive to changes in the concentration of the acid. Strong acids and bases beyond slight amounts totally inhibited growth. Electrolytes added to a culture medium may influence growth through the chemical effect of their ions and molecules or by altering the reaction. From the small amount of evidence which Molisch and Jost have obtained, we cannot definitely assign the favorable effects of the acids used to either category. If, as it appears with *Rhododendron*, both malic and citric acid promote growth, their effect may reasonably be considered as due to a favorable adjustment of the reaction.

Our experiments to determine the effect of changes in pH on pollen-tube growth were unsuccessful. Very little or no growth could be obtained in the presence of acids and bases used, and the trials were abandoned. The culture medium used in the majority of the writer's other experiments, consisting of shred agar, distilled water, commercial cane sugar, and a small amount of sterile yeast, gave a reaction of about pH 6.6. When the shred agar was replaced by "Bactoagar," it was found that the acidity was considerably increased. Standards were not at hand for making determinations below pH 6.0, but the "Bactoagar" medium was at least as acid as this. A sample of gelatin which also failed to promote good growth was found to have an acidity of pH 6.0 or less.

As shown in table 8, the culture media containing "Bactoagar" gave lower germination and less than one fifth the amount of growth that was obtained when shred agar was used. According to Fellers (1916), "Bactoagar" is somewhat lower in ash content than the cruder product. Nothing is given, however, regarding the composition or proportions of the mineral constituents. Possibly these remaining salts are responsible for the toxic effect of the "Bactoagar," but on the basis of the facts at hand we are inclined to attribute its failure to promote growth to the high acidity found.

The most interesting fact revealed in our experiments was that cultures containing shred agar gave about 17 percent more growth with commercial cane sugar than with chemically pure sugar. This fact may be significant; at least it suggests a method of attack on the problem of the toxicity of salts to pollen tubes that has proved fruitful in studying their rôle in the physiological processes of various other forms. According to Fellers (1916), ordinary shred agar contains about 3.33 percent ash; the cane sugar of the trade contains slight amounts of mineral impurities. On the addition of chemically pure sucrose, shred-agar cultures give, as shown in table 8, an amount of growth equal to 85; when commercial cane sugar *with its mineral impurities* is substituted for chemically pure sugar, the amount of growth is increased to 100. The content of mineral material is undoubtedly variable in both agar and commercial sugar, though probably seldom over 0.5 percent in the latter. We are in the dark as to the chemical nature of these salts; hence any conclusion as to their mutual effect must be tentative

only. But the facts as far as they go indicate antagonism; in other words, the agar contains substances whose injurious effects as revealed by decreased growth when used with pure sucrose are diminished by the mineral impurities in the commercial cane sugar. Whether or not such is actually the case is a question that must await the results of more detailed investigation, but the suggestion is put forward at the present time as a working hypothesis.

We are in urgent need of further knowledge of the effects of salts on pollen-tube growth. As the extensive researches of Loeb (1906, 1912), Osterhout (1922), and others have shown, these play a cardinal rôle in determining the permeability of the cell membrane. If the technic of pollen culture *in vitro* can be so adapted as to permit a study of some of the conditions influencing permeability of the protoplasmic membranes of pollen and pollen tubes, much additional light may be thrown upon the problems of toxicity of salts, bursting, and the growth processes in general. Indeed, it is not entirely improbable that the slight amount of growth secured with many forms on our present artificial media is due to exosmosis of essential salts normally retained within the pollen tube.

# CYTOLOGICAL STUDIES IN THE GENUS CRATAEGUS<sup>1</sup>

ALBERT E. LONGLEY

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An interesting account of the changes the genus *Crataegus* has undergone in the last sixty years is given by Brown (3). In 1857, when Gray's "Field, Forest, and Garden Botany" was published, there were only 10 species and 4 varieties, while Focke in Engler and Prantl's "Die natürlichen Pflanzenfamilien" gives only 30 or 40 species for the whole north temperate zone. A sign of increase was apparent in Chapman's "Flora of the Southern United States," published in 1897, which gave 15 species for its range. Since 1900, however, the increase in the number of species has been striking. Britton's "Manual," in 1901, for the northern states, west to the hundredth meridian, records 31 species; Small's "Flora of the Southern United States," in 1903, gives 185 species; Sargent's "Trees of North America," in 1905, describes 132 species; while in Gray's "New Manual," published in 1908, 65 species and 50 varieties are described. By 1910 there had been listed 750 species, and in the last twenty years Sargent alone has described approximately 400 species.

Has hybridization caused species-multiplication in this genus? is the question asked by Brown. Standish (21) has shown that reproductive sterility is common in our native *Crataegi*. This phenomenon is generally considered a character peculiar to hybrids. Recently it has been shown that polyploidy and polyspory characterize many hybrids and are associated with reproductive sterility.

The present work was undertaken to determine if, in this polymorphic genus, polyploidy and polyspory exist. Meyer (14) studied in connection with his work on graft hybrids the number of chromosomes in the pollen mother cells. He found the diploid number to be 32 and the haploid 16 in *Crataegus monogyna*.

## MATERIAL AND METHODS

The material used was collected at the Arnold Arboretum of Harvard University from accurately labeled trees on the hawthorn hillside, a collection which has been gathered and studied specially by Professor Sargent during the past thirty years.

The buds were collected on warm days, cut open, and put immediately into a weak chromoacetic killing fluid. They were imbedded in both paraffin and nitro-cellulose; the latter proved to be more satisfactory. Several stains were used, but Heidenhain's iron-alum haematoxylin gave the best results.

<sup>1</sup> Contribution from the Laboratories of Plant Morphology of Harvard University.

The material was examined to get as many stages of the pollen-mother-cell development as possible, using a 1.5 mm. Zeiss apochromatic objective and a no. 12 compensation ocular.

### SECTION I

There is no recent monograph of our North American *Crataegi*, and consequently I have resorted to Sargent (20) and to a classification sheet of W. W. Eggleston for the names and classification of the *Crataegi* described in the following section. Sargent has made a study of this genus for the last thirty years, and Eggleston (7) shows that he has studied in detail this perplexing genus since June, 1899.

Before entering upon the detailed discussion of the cytological study that I have made of approximately 100 species of *Crataegus*, I shall refer to some features characteristic of this genus as a whole.

In the first place I would stress the advantage of studying the uncut cells. I have found that sections 15 microns thick cut from material imbedded in parlodion are as clear as sections half as thick cut from material imbedded in paraffin, and, for the type of cytological work that I have attempted, I find the first-mentioned method to be much superior.

There are present, very generally, in the cytoplasm of the pollen-mother-cells of this genus, masses of material staining black or dark blue with haematoxylin. The presence of such masses was found to be very confusing in my study of *Rubus* material, but it is even more confusing in this genus, for it does not entirely disappear even at the metaphase as I found to be the case in *Rubus*.

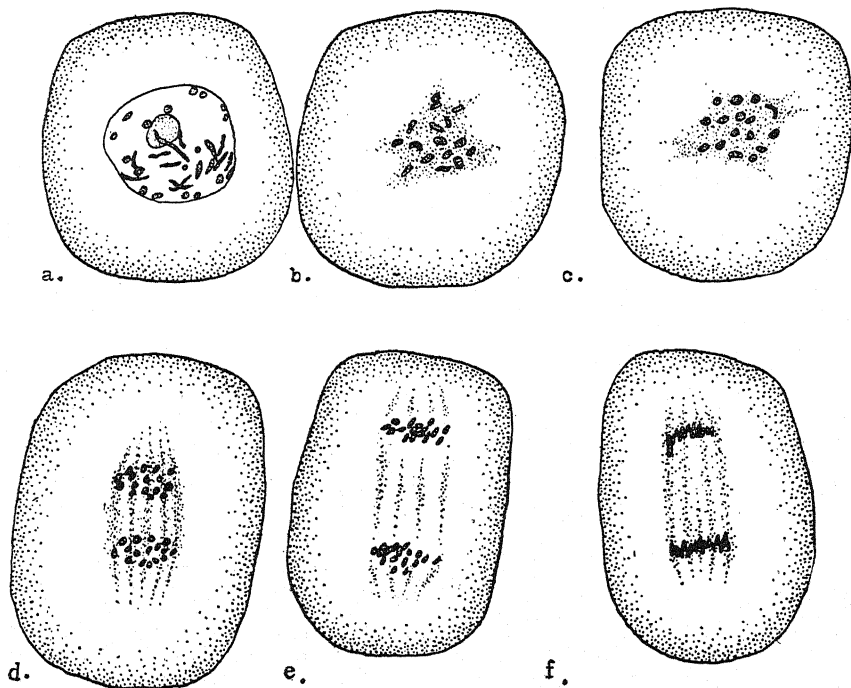
Another difficulty encountered was the failure of the chromosomes to pair until the late prophase, and consequently many of the chromosome counts had to be made at the metaphase and anaphase stages, which are often unfavorable unless the chromosomes are caught in an uncrowded condition. A third and ever-present difficulty is the smallness of the elements for detailed examination. Even with the best lenses obtainable, the chromosomes seem minute and the study of details is exceedingly tedious.

### Diploid *Crataegi*

Miss Standish (21) has shown that about one fifth of the *Crataegi* she studied were characterized by morphologically good pollen. My study of this genus has led to the discovery of 13 diploid species or about one seventh of all the forms studied. The method used in collecting may, in some degree, account for the small proportion of diploid forms, for I collected extensively of the *Intricatae*. This group, Standish has pointed out, is composed of probable hybrid forms.

*Punctatae**C. Collina* Chapm.*C. punctata* Jacq.

The development of the pollen mother cell of *C. collina* is represented in Plate XVI. Figure 1 pictures the late prophase; the nucleolus is still faintly present and the bivalent chromosomes are scattered at the periphery of the nucleus. A study of many stages favorable for chromosome-counting showed clearly that the gametophytic number is 16, and from this it seems safe to assume that the sporophytic number of diploid species is 32. That species possessing 16 bivalent chromosomes are true diploid species is substantiated by the fact that in no case are there indications of irregularity in chromosome-distribution during the heterotypic and homoeotypic divisions. The even and equal distribution has been an aid in determining the status of a form in cases in which it was difficult to get clear and indubitable chromosome counts. Following the further development of this species, figure 2 represents the early metaphase; the spindle is clearly multipolar, such as has been described by Osterhout (17) and others: the chromosomes are at this stage widely separated and in favorable positions for counting.



TEXT FIG. 1. Early stages in the development of the pollen grains of a few diploid species of *Crataegus*: a, prophase of *C. monogyna*; b, early metaphase of *C. Treleasei*; c, early metaphase of *C. mollis*; d-f, anaphases of *C. tomentosa*, *C. sanguinea microphylla*, and *C. cuprea*.

Figure 3 represents the late metaphase, and the point of interest at this stage is that all chromosomes are in a compact mass at the nuclear plate. This and the stage pictured in figure 4 are important by reason of the regular distribution of the chromosomes. In the latter figure, the chromosomes are moving towards the poles in two masses. Figure 5 pictures the nuclei at interkinesis; no definite number of chromosomes can be made out at this stage, and the presence of a nucleolus indicates that the nucleus is in the resting stage. The presence of resting nuclei at interkinesis, perhaps, can be attributed to the fact that, at the time of the development of the pollen mother cells, growth is interrupted by frequent changes in temperature, and consequently the two reduction divisions do not follow in rapid succession. Figures 6-10 represent the homoeotypic division of this species. It is carried out in a perfectly regular manner, with an occasional opportunity to determine the chromosome number at the early metaphase or late anaphase. The result of such a regular division is the production of four normal pollen grains. The second species, *C. punctata*, was not so favorable for study, but showed no deviation from the conditions just outlined.

#### *Rotundifoliae*

##### *C. Margaretta* Ashe.

Counts of the chromosomes were made for this species at the early metaphase of the heterotypic division; the number was found to be 16. The divisions were in every sense regular, and so, after studying clear figures as represented in text figure 2, *a*, the heterotypic prophase, I was assured that this species is diploid.

#### *Molles*

##### *C. mollis* Scheele.

##### *C. Treleasei* Sarg.

##### *C. sera* Sarg.

I have found more than the usual proportion of diploid forms in this group. The three species listed above show no irregularities in the developing pollen mother cell, and I have made many counts of the chromosomes in the early heterotypic metaphase and found the number to be 16. Text figure 1, *b* represents such a stage in *C. Treleasei* while text figure 1, *c* pictures the same stage for *C. mollis*, and there seems to be no doubt that these species are diploid.

#### *Macracanthae*

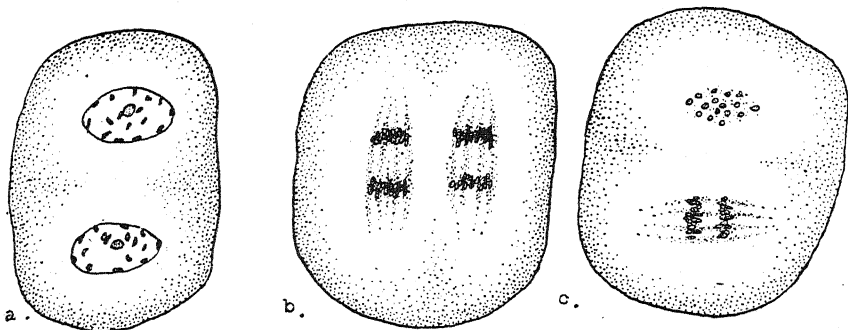
##### *C. tomentosa* L.

This species is characterized by very regular divisions, and the small amount of chromatin material is almost sufficient to justify the opinion that this is a diploid form. Text figure 1, *d* represents the heterotypic anaphase, when the two sets of chromosomes are seen on their way to the poles.



*Virides**C. nitida* Sarg.

I was able to determine the chromosome number for this species in the late heterotypic prophase and in the early heterotypic metaphase, and so I do not hesitate in classing it as a diploid species. Text figure 2, *c* represents



TEXT FIG. 2. Stages in the homoeotypic divisions in three diploid forms: *a*, prophase of *C. Margareta*; *b*, anaphase of *C. Canbyi*; *c*, anaphase of *C. nitida*.

the homoeotypic metaphase; in the upper spindle the chromosomes are separated sufficiently to count the 16. The lower spindle is in the early anaphase and shows the regular separation of all chromosomes.

*Intricatae**C. cuprea* Sarg.

This species is an exception in the *Intricatae* group, for it is the only one that showed the heterotypic anaphase to be regular. Text figure 1, *f* pictures such a stage; the chromosomes are in a compact mass and move as one unit towards either pole. The chromosome number was determined in the heterotypic prophase and found to be 16.

*Crus-galli**C. Canbyi* Sarg.

The *Crus-galli* group is characterized by the ease with which the chromosomes take a haematoxylin stain. I found only one species that showed no irregularities in the reduction divisions, and that has 16 bivalent chromosomes. Text figure 2, *b* represents the homoeotypic anaphase of *C. Canbyi*; there is no sign of chromosomes in the cytoplasm left over from the previous division, and no sign of irregularity in the separation of the chromosomes.

*Oxyacanthae**C. Oxyacantha* L.*C. monogyna* Jacquin.

My material of these two species was mostly too young, and so my preparations lack some of the critical stages, especially in *C. Oxyacantha*, though

the few stages of the heterotypic division hit upon were strikingly regular. In *C. monogyna* the chromosome number was determined with certainty and found to agree with that reported by Meyer (14). I have pictured the unpaired chromosomes in text figure 1, *a*, where the 32 univalents can be counted and in several cases the pairs can be picked out.

*C. sanguinea microphylla* Schrad.

This and the two *Oxyacantha* species are exotic. I was able to determine definitely the chromosome number, and text figure 1, *e* shows the chromosomes sufficiently separated for counting. The 16 chromosomes show a perfect regularity in their distribution to the four daughter nuclei.

**Triploid Crataegi**

Miss Standish (21) has separated the Crataegi with imperfect pollen into two classes. I have separated species showing irregularities in the distribution of chromosomes according to the number of chromosomes found to be present. The triploid group includes three quarters of the species studied, and in this group there are marked variations in chromosome distribution. I have reserved a few extreme cases to be treated at the close of this section; the descriptions below are of representative species to illustrate the wide occurrence of the triploid condition and the general behavior of chromosomes. New difficulties arise with an increase in chromosome number, and associated with this increase is the irregular distribution of chromosomes; so counts for the most part are restricted to early stages in the heterotypic divisions, the number of chromosomes at this stage being less open to question. It is necessary to state that a few species have been included in the triploid group because of their general behavior, and I was able to make only an approximate chromosome count. These are indicated in the description below.

*Pruinosae*

*C. cognata* Sarg.

*C. delawarensis* Sarg.

*C. fusca* Sarg.

*C. pruinosa* C. Koch.

This group, according to Standish (21), shows diversity in pollen conditions. I was unable to obtain sufficient material to determine the chromosome number of the two forms she reported as having good pollen. I have chosen one of the species that showed 60 percent imperfect pollen to illustrate the conditions found commonly in all triploid Crataegi.

Plate XVII represents stages in the development of the pollen mother cell of *C. pruinosa*. Figure 1, the late prophase, shows the 24 diploid chromosomes at the periphery of the nucleus. Such stages are quite difficult to find due to the lateness of the pairing of the univalents. The heterotypic metaphase was found favorable for counting the chromosomes and so was quite frequently studied and pictured. Figure 2 represents the late meta-

phase; some of the chromosomes are tardy in reaching the nuclear plate, a condition that is typical of forms having more than 16 chromosomes. Figures 3 and 4 represent the early and late anaphases. The last figure shows to what degree the chromosomes lag on the spindle. Such a condition as that just cited involves the extrusion of chromosomes into the cytoplasm. Figure 5 pictures a very early telophase. The chromosomes in this case have not lost their individuality, and counts showed variations due to the loss of chromosomes that have lagged and are consequently scattered in the cytoplasm. Figure 6 is a more typical telophase. The nucleus at interkinesis is generally in the resting condition, and it is rare to find such a phase as that pictured in figure 5. The homoeotypic division is often much more regular than the heterotypic division, but in this species lagging chromosomes are found on the spindle in abundance in both reduction divisions. Figure 7, the homoeotypic metaphase, shows the chromosomes extruded into the cytoplasm. These are often lost to view in the interkinesis, but appear again at the homoeotypic metaphase. In rare cases, these extruded chromosomes play a rôle in the formation of dwarf pollen grains. Figures 8 and 9 represent the homoeotypic anaphase. In both figures chromosomes are lagging on the spindle, and in the homoeotypic telophase these chromosomes are scattered in the cytoplasm. This species shows no polyspory; the chromosomes in the cytoplasm degenerate so that four pollen grains result, but with varying chromosome number in each nucleus.

The remaining species listed are triploid forms, all showing striking irregularities in the heterotypic anaphase. *C. delawarensis* gave some very favorable figures for chromosome determination. Text figure 3, *d* represents a heterotypic anaphase in which the chromosomes are widely separated and can be readily counted. The number is distinctly 24, and the separation is irregular, but all chromosomes eventually reach the polar region and so are only rarely found in the cytoplasm.

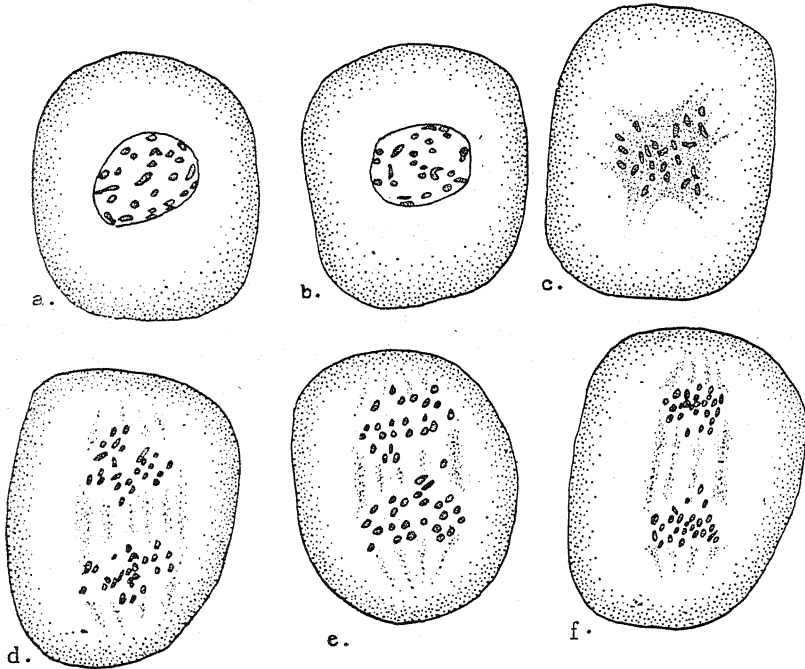
#### *Punctatae*

*C. pausiaca* Ashe.

*C. vicina* Sarg.

Text figure 3, *e* represents a very favorable homoeotypic anaphase of *C. pausiaca* in which the chromosome number can be definitely determined; some are lagging, a condition that is very common in this species. 24 chromosomes were also distinctly counted in the heterotypic anaphase, making more certain the status of this species. *C. vicina* shows an accentuation of the irregularities found in *C. pausiaca*. Plate XVIII represents some of the significant stages in pollen-formation in *C. vicina*. Figure 1 represents a characteristic heterotypic metaphase; the spindle is distinctly multipolar, and the 24 chromosomes are separated sufficiently to be readily counted. Figures 3 and 4 represent characteristic anaphases: chromosomes

are lagging on the spindle, and the extrusion of a few gives figures such as are represented in figure 5. Figure 6 pictures the late homoeotypic metaphase; most of the chromosomes are collected at the nuclear plate, but a few are seen in the cytoplasm, having been extruded during the heterotypic division. Figures 7 and 8 represent stages in which we see the formation of



TEXT FIG. 3. Stages in the heterotypic divisions in a few triploid forms: *a, b*, diakinesis of *C. Bisselii* and *C. paucispina*; *c*, early metaphase of *C. exclusa*; *d-f*, anaphases of *C. delawarensis*, *C. pausiaca*, and *C. Delosii*.

four pollen grains produced from one pollen mother cell in spite of the irregularities found in the early stages of development. There were anthers that showed conditions such as I have pictured in figures 9 and 10, mother cells that have five or six nuclei, one or two being dwarf. As a consequence, instead of the normal tetrad five or six pollen grains are produced, four of them being nearly normal in size, the other one or two being dwarf.

The Punctatae group was favorable for study; in five or six species I found the widest range of conditions. *C. collina* is perfectly regular in its chromosome distribution, and *C. vicina* is so irregular that quite frequently polysypis is found.

#### Coccineae

*C. assurgens* Sarg.  
*C. Eamesii* Sarg.  
*C. flabellata* Sarg.

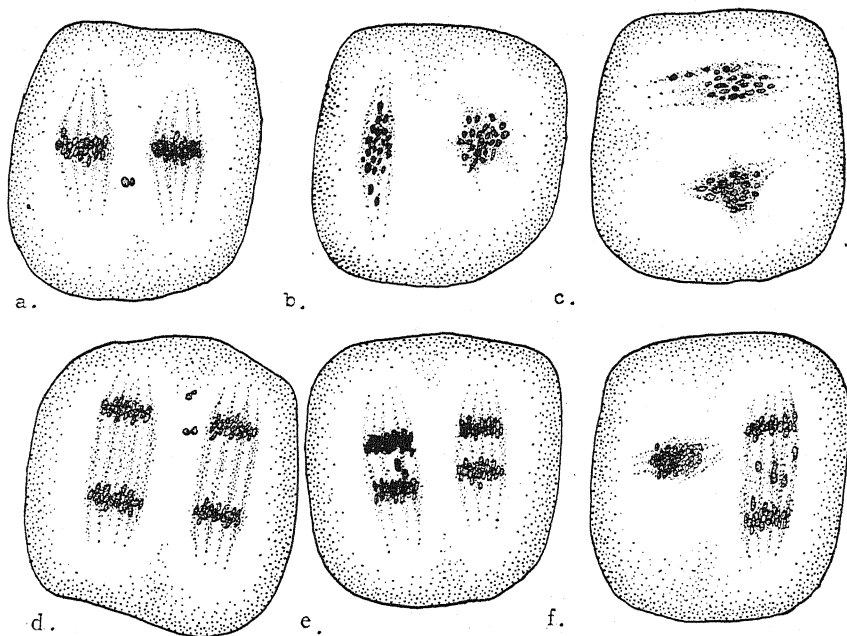
*C. exclusa* Sarg.  
*C. fluvialis* Sarg.  
*C. Holmesiana* Ashe.

*C. Hillii* Sarg.  
*C. Pringlei* Sarg.  
*C. sertata* Sarg.

*C. lobulata* Sarg.  
*C. pedicillata* Sarg.  
*C. tardipes* Sarg.

I have found that variation in the chromosome-distribution is general in this group. There was some difficulty in determining the chromosome count for some of the species, in view of the small size of the elements, but, after a study of many figures, there are only a few in which I hesitate in assigning 24 as the gametophytic chromosome number. Text figure 3, *c* represents a spindle in the multipolar stage, showing distinctly 24 chromosomes scattered on it, from the species *C. excluda*.

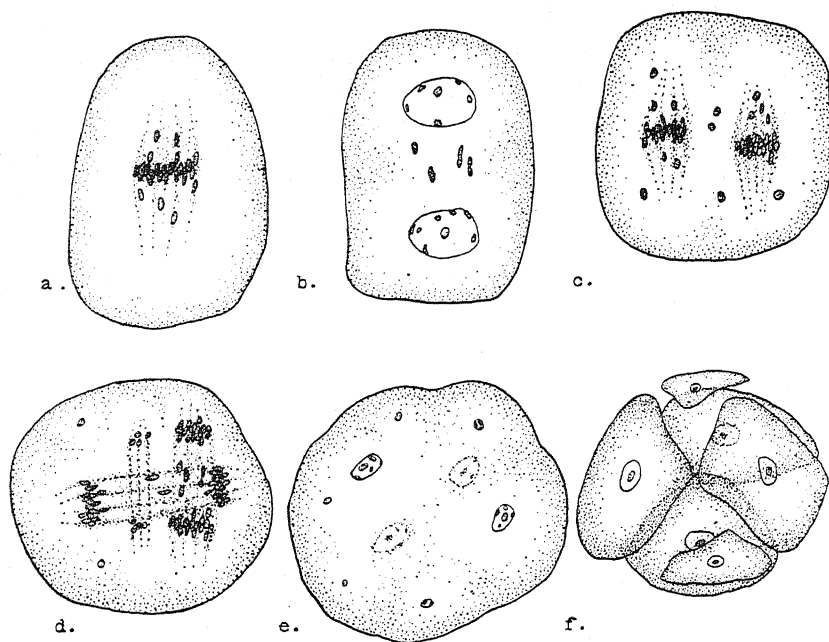
A representation of a typical homoeotypic metaphase of *C. Eamesii* is seen in text figure 4, *b*; the chromosomes can be counted, and the spindle to the left is faintly multipolar. Text figure 4, *c* represents the homoeotypic metaphase of *C. fluviatilis*, and the condition is a duplication of that pictured



TEXT FIG. 4. Stages in the homoeotypic divisions in six triploid forms: *a-c*, metaphases of *C. Palmeri*, *C. Eamesii*, and *C. fluviatilis*; *d-f*, anaphases of *C. Wheeleri*, *C. Buckleyi*, and *C. Jackii*.

for *C. Eamesii*. A species of this group that has received special attention is *C. Hillii* on account of the very general occurrence of polyspory. Text figure 5 represents some characteristic conditions found in the pollen-formation of this species. Figures *a* and *b* represent conditions common to practically all triploid forms, but the extrusion of chromosomes at the hetero-

typic division is very general and leaves the cytoplasm dotted with chromatin material. Figure *c* represents the homoeotypic metaphase; at this stage can be seen chromosomes in the cytoplasm and others on the spindle, but lagging outside the nuclear plates. Figure *d* represents the late anaphase; there can be seen the two major spindles and a dwarf spindle with a few additional chromosomes in the cytoplasm. There seem to be more chromosomes lagging on the spindle than is usual in the homoeotypic anaphase; for often the homoeotypic division is quite regular even in forms showing striking irregularities in the heterotypic division. Figure *e* represents a pollen mother cell before the cell walls are laid down. There can be seen 4 major nuclei, 2 dwarf nuclei, and three small chromatin masses in the cytoplasm. The multinucleated mother cells lead to the condition pictured in figure *f*, where there are 6 pollen grains, two of which are distinctly dwarf.



TEXT FIG. 5. Stages in the production of polypspory in *C. Hillii*.

### *Intricatae*

*C. Bealii* Sarg.  
*C. Boyntonii* Beadle.  
*C. dacroides* Sarg.  
*C. flavida* Sarg.  
*C. inducta* Ashe.  
*C. intricata* Lange.  
*C. meticulousa* Sarg.

*C. Bisselii* Sarg.  
*C. Buckleyi* Beadle.  
*C. Delosii* Sarg.  
*C. foetida* Ashe.  
*C. infera* Sarg.  
*C. modesta* Sarg.  
*C. Painteriana* Sarg.

*C. pinetorum* Beadle.  
*C. padifolia* Sarg.  
*C. Sargentii* Beadle.  
*C. vericunda* Sarg.

*C. pusilla* Sarg.  
*C. Stonei* Sarg.  
*C. Wheeleri* Sarg.

This group was studied in detail in order to test the statement of Standish (21) that the forms representing this group which she studied *are strikingly sterile*. I do not hesitate to include the above-named species, on the basis of detailed cytological examination, in the group of triploid Crataegi. Most counts were made at the early heterotypic metaphase, which is one of the most favorable stages. The conditions found in the many species of this group are so similar that I have made figures of only a few representative species. Text figure 3, *f* represents the heterotypic anaphase of *C. Delosii*; the chromosomes are scattered so that the 24 at each pole can be counted. Text figure 3, *a* represents the late prophase of *C. Bisselii*. Whenever this stage was found, the 24 chromosomes stood out clearly. Text figure 6, *b* pictures a significant stage in the pollen-formation of *C. Sargentii*; a large number of chromosomes are lagging on the spindle, and some give promise of being extruded into the cytoplasm. Text figure 4, *d* represents the condition in *C. Wheeleri* at late homoeotypic anaphase; the homoeotypic divisions are regular, but in the cytoplasm is seen the shadow of chromosomes extruded during the previous heterotypic division. Text figure 4, *e* represents an early homoeotypic anaphase of *C. Buckleyi*; there are chromosomes lagging on the spindle, a phenomenon that is met with less frequently than in the heterotypic anaphase. In the Intricatae, triploid forms are the rule, and associated with this increase in number of chromosomes above the diploid number is a general irregularity in the distribution of the chromatic material.

#### *Tenuifoliae*

*C. paucispina* Sarg.  
*C. tarda* Sarg.

*C. pentandra* Sarg.

The forms of this group are favorable for determining the chromosome number, and I have found that all species studied are triploid. Text figure 3 *b* represents the late prophase of *C. paucispina*; the 24 chromosomes can be counted readily.

#### *Rotundifoliae*

*C. Brunetiana* Sarg.  
*C. Jackii* Sarg.

*C. Dodgei* Ashe.

The three species named above show the characteristics common to triploid Crataegi. Text figure 4, *f* represents a typical homoeotypic anaphase of *C. Jackii*; the spindle to the right shows chromosomes lagging on the spindle. It is difficult at this stage to determine the chromosome number, but a close approximation can usually be made.

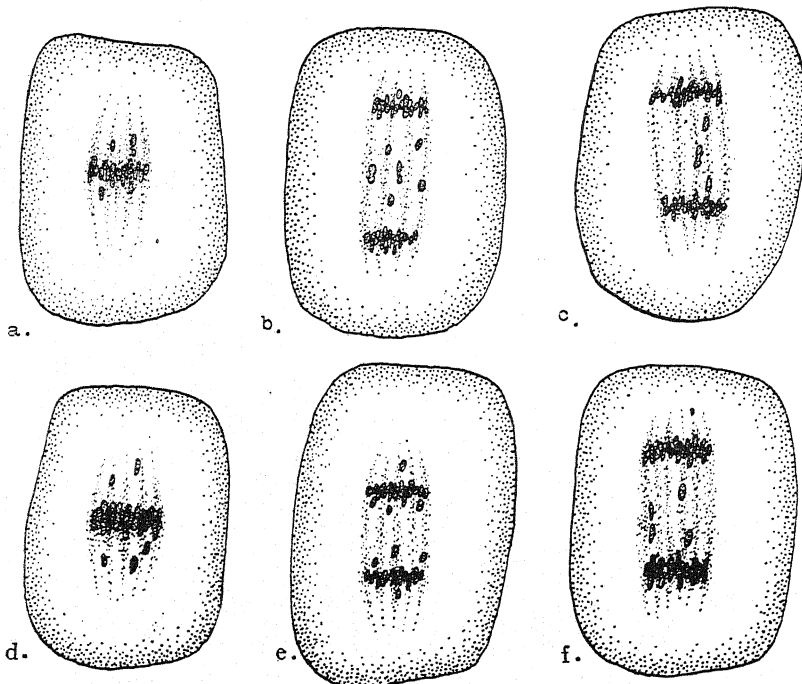


*Molles**C. Arnoldiana* Sarg.*C. diffusa* Sarg.*C. lanuginosa* Sarg.

Text figure 6, *a* represents the late heterotypic metaphase of *C. lanuginosa*, a triploid species of this group. The chromosomes are shown lagging in their movement towards the nuclear plate.

*Crus-galli**C. Crus-galli* L.*C. phlebodia* Sarg.*C. Palmeri* Sarg.*C. rivalis* Sarg.*C. rotunda* Sarg.

I found that the chromosomes in this group stained deeply with haematoxylin and consequently stood out sharply in all preparations. The chromosomes are in a compact mass at the nuclear plate, and so studies were confined to the heterotypic prophase and early heterotypic metaphase. Text figure 6, *d* represents a late heterotypic metaphase of *C. Crus-galli* showing chromosomes lagging in their movement towards the nuclear plate. Text figure 4, *a* represents a homoeotypic metaphase of *C. Palmeri*; the



TEXT FIG. 6. Stages in the early divisions in triploid forms: *a* and *d*, metaphases of *C. lanuginosa* and *C. Crus-galli*; *b*, *c*, *e*, and *f*, anaphases of *C. Sargentii*, *C. Chapmanii*, *C. meticulosa*, and *C. Douglasii*.

nuclear plate has been formed, but a sign of irregularity in the heterotypic division is seen in the presence of two chromosomes in the cytoplasm.

*Asperifoliae*

*C. asperifolia* Sarg.

*Douglasianae*

*C. Douglasii* Lindl.

*Dilatatae*

*C. coccinoides* Ashe.

*Macracanthae*

*C. Chapmanii* (Beadle) Ashe.

The forms listed above are individual examples of triploid species found in widely separated groups. I collected with the idea that by choosing a species with a wide range and another with a local range I would find very different cytological conditions. I have found that irregularities are more striking in forms with a very local range, but I have found that even many forms with a wide range are triploid or even tetraploid.

Text figure 6, *f* represents a critical stage in the pollen-mother-cell development of *C. Douglasii*, showing chromosomes left on the spindle after the main masses have reached the poles. Text figure 6, *c* is a heterotypic anaphase of *C. Chapmanii*. These examples go to show that triploid species are not confined to such groups as the Coccineae and Intricatae, but are general in their distribution.

*Intricatae*

**Tetraploid Crataegi**

*C. apposita* Sarg.

*C. Peckii* Sarg.

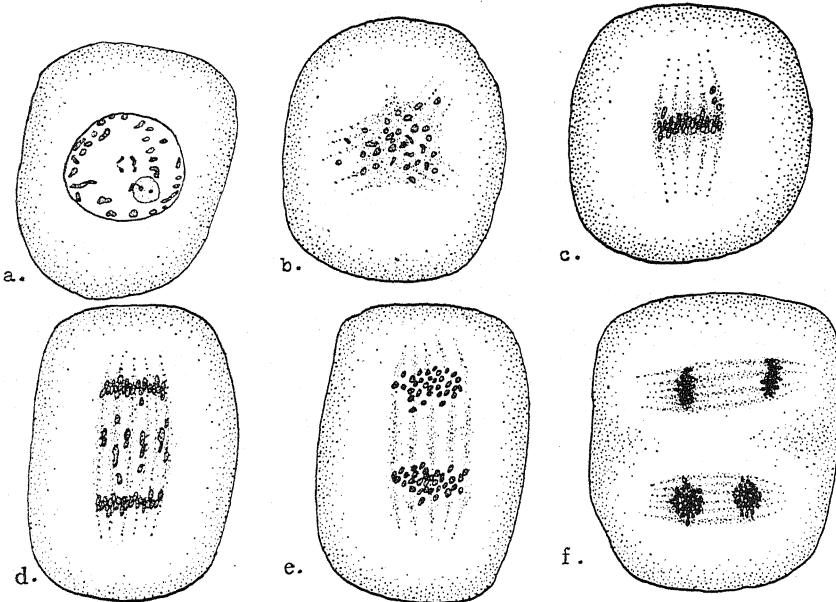
*C. Bartoniana* Sarg.

The number of tetraploid Crataegi is small, and I have taken care to make certain that 32 gametophytic chromosomes are present.

Text figure 7, *b* represents a favorable stage for determining the chromosome number of *C. apposita*, which is clearly 32. This species shows very few irregularities in the distribution of the chromosomes during the reduction divisions. A characteristic homoeotypic anaphase is represented in text figure 7, *f*. Text figure 7, *e* pictures a favorable stage in *C. Peckii* for determining the chromosome number, and in this species also irregularities in chromosome distribution are few. The third form, *C. Bartoniana*, is a species in which irregularities are very frequent in their occurrence and occasionally lead to polypory.

*Rotundifoliae**C. rotundifolia* Moen.*C. rotundifolia* var. *pubera* Sarg.

The chromosome counts in these two species were made at late prophases, and text figure 7, *a* represents such a view in *C. rotundifolia* var. *pubera*. The chromosomes can be counted in their scattered position about the



TEXT FIG. 7. Stages in the divisions in tetraploid forms: *a*, prophase of *C. rotundifolia* var. *pubera*; *b*, early metaphase of *C. apposita*; *c*, late metaphase of *C. rotundifolia*; *d*, irregular anaphase of *C. Forbesae*; *e*, regular anaphase of *C. Peckii*; *f*, regular homoeotypic anaphase of *C. apposita*.

periphery of the nucleus. Associated with the tetraploid chromosome number are slight irregularities in chromosome-distribution, as pictured for *C. rotundifolia* in text figure 7, *c*, where the chromosomes are lagging in their movement into the nuclear plate.

*Tenuifoliae**C. Forbesae* Sarg.

This species is marked by striking irregularities in chromosome distribution such as are illustrated in text figure 7, *d*, a very characteristic heterotypic anaphase. Such irregularities often lead to the extrusion of chromatin material, and such material is frequently seen in the cytoplasm during the homoeotypic division. One might expect to find polyspory frequently in a form showing such irregularities.

### Tetraploid and Triploid *Crataegi*

I stated earlier that Standish (21) reported species with complete abortion of pollen, and the following-named species can certainly be included in this class. It was only after cutting many buds that I was able to find anthers containing pollen mother cells, and still more rarely anthers containing mother cells in the process of reduction.

#### *Intricatae*

*C. pallens* Beadle.

*C. pygmaea* Sarg.

#### *Uniflorae*

*C. Smithii* Sarg.

#### *Triflorae*

*C. triflora* Chapm.

#### *Molles*

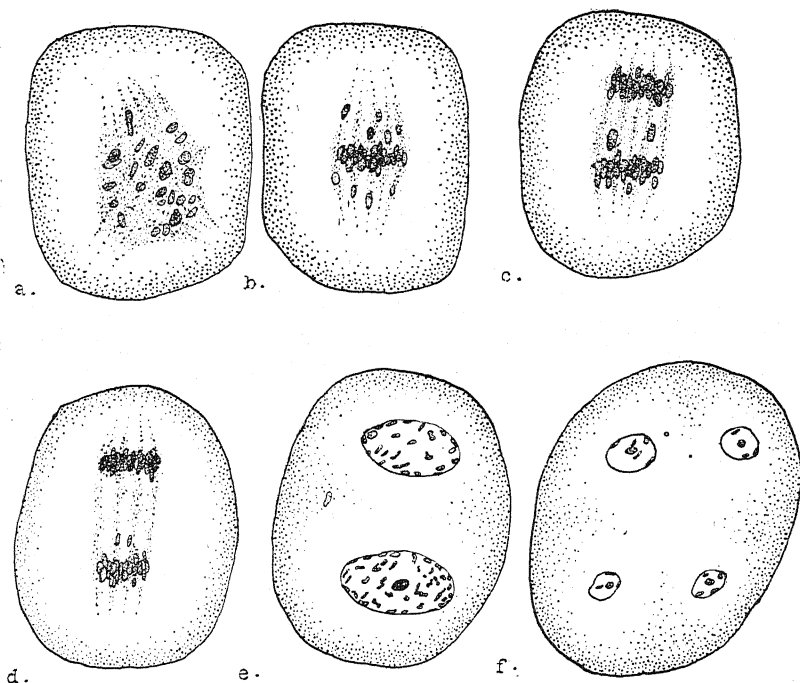
*C. disperma* Ashe.

Text figure 8 has been prepared to illustrate some stages in the development of the pollen mother cells in this group that show early degeneration of the male cells. It cannot be said that the text figure represents typical stages, but rather shows that in the few cells in which division phases were found they are very little different from other triploid and tetraploid *Crataegi*.

Text figure 8, *a* represents an early metaphase of *C. triflora*. The spindle is multipolar, and the chromosomes stand out very distinctly. Later development shows lagging and extruded chromosomes, but more striking is the vacuolated appearance of the cells. Text figure 8, *b* represents the late heterotypic metaphase in *C. Smithii*, the first species in which I found pollen mother cells that stained weakly and had a strikingly impoverished appearance. I was able to trace the development of the tetrad, and both heterotypic and homoeotypic divisions showed extrusion of chromosomes or of chromatin masses. I found that, as the cell approached the last stages of degeneration, the chromatin material was affected; it clumped together and did not seem to form chromosomes. At division these clumps divide very irregularly, often simulating amitotic stenosis, and a connecting strand gives the chromatic material a very characteristic dumbbell-shaped appearance. Figure *f* represents a late homoeotypic telophase of *C. Smithii*. This stage is sometimes reached, though more frequently the cell seems to collapse before arriving at this advanced stage of division.

Text figure 8, *c* represents the late anaphase of the heterotypic division of *C. pallens*, a form in which I have determined the chromosome number and found it to be 32. Irregularities are very abundant in the anaphases of both the heterotypic and the homoeotypic divisions, if the pollen mother cell develops to these stages. The typical pollen mother cell of this group

of *Crataegus* is deficient in cytoplasm, the whole cell seems thin and vacuolated, the chromosomes stand out strongly, and the cell seems to lack strength to carry it to the tetrad stage. *C. pygmaea*, another of the Intricatae, is a triploid species, and figure *e* represents an interkinesis. On the left of the cell is seen extruded chromatin material, the result of an irregular heterotypic anaphase.



TEXT FIG. 8. Divisions in a few almost completely sterile forms: *a*, heterotypic of *C. triflora*; *b*, late heterotypic metaphase of *C. Smithii*; *c*, heterotypic anaphase of *C. pallens*; *d*, heterotypic anaphase of *C. disperma*; *e*, interkinesis of *C. pygmaea*; *f*, tetrad before cell-plate-formation of *C. Smithii*.

*C. disperma* showed more rarely the phenomenon described for the previously mentioned species. It was not difficult to find normal-appearing tetrads, but many of the anthers showed mother cells that were thin and broke down before the reduction was complete. It might be said to be intermediate between triploid species such as *C. Smithii* and the more fertile *C. Pringlei*.

The significance of the three natural divisions into which *Crataegi* can be separated, after a cytological study of the developing mother cells, I shall leave for a more detailed consideration in the concluding part of this paper.

## SECTION II

In an article on the genus *Rubus*, in the previous issue of this JOURNAL, I have reviewed the investigations on plants showing reproductive sterility, polyploidy, and polyspory. This review made prominent the relation between the above-mentioned characters and the genetic impurity of the species under consideration.

Reproductive sterility in animals is the direct result of hybridization. A few such cases have been given a critical cytological examination. Wodse-dalek (24) has worked out the cause of sterility in one of the oldest known hybrids, the mule. He found reduction of the 51 chromosomes incomplete, giving bivalent and univalent chromosomes in varying numbers. Abnormalities in mitosis occur invariably in primary spermatocytes that attain the metaphase stages. There seems to be an attempt at this stage to eliminate some of the chromatin material. Disintegration as well as abnormalities seem to be restricted to the primary spermatocytes, most cells disintegrating during the prophase. Others meet this fate in the metaphase and early anaphase. The remaining few that survive the metaphase succumb soon after, and the hybrid remains sterile. These conditions are very nearly duplicated in reduction divisions of sterile plant hybrids, such as Gregory (10) reports for crossed peas in which he finds that often the cells degenerate without coming to the heterotypic metaphase.

Guyer (12) has described the spermatogenesis of normal and hybrid pigeons. He finds that, from forms not too distantly related, fertile hybrids are easily obtained, but infertile hybrids result from crosses of very distinct and separate species. The latter are more difficult to secure.

Federley (8) describes, in his work on hybrid forms of *Pygaera*, conditions very similar to those described by Rosenberg (19) for the genus *Hieracium*. He found one class with both univalent and bivalent chromosomes present, the former causing striking irregularities. In another class, the chromosomes do not collect at the nuclear plate, and the resulting daughter cells have very unequal chromosome numbers. In a third type, the division is typically somatic, no reduction in chromosome number occurring.

Baltzer (1) has shown in his work on echinoderm hybrids that not only do species of these cross readily, but also that associated with such crosses is a failure of chromosomes from the two parents to mix freely. This condition was first brought out by Moenkhaus (15) in a cross between *Fundulus heteroclitus* and *Menidia notatus*, in which, in the early divisions of the egg, the long chromosomes derived from the *Fundulus* parent remained separate and distinct from the short *Menidia* chromosomes for several divisions. Morris (16) found a similar lack of assimilation in *Fundulus*-*Ctenolabrus* hybrids. Finally, Pinney (18), studying teleost hybrids, found abnormalities in chromosome behavior including elimination, fragmentation, and abnormal distribution. The study of these hybrids was confined to young embryos,

for all failed to mature and so there was no opportunity to determine the nature of the reduction phases in these bigeneric hybrids.

I return to a consideration of the Rosaceae, and before proceeding to a discussion of the genus *Crataegus* I shall refer briefly to two papers on cytological studies of the genus *Rosa*.

Blackburn and Harrison (2) assign, with no hesitation, the cause of irregularity in chromosome-distribution and of the resulting defective pollen to hybridity.

Täckholm (22) found, in his study of roses, that diploid species are segregated in certain sections of the genus. Other sections are composed of diploid and polyploid forms, and, finally, the Canina section shows not a single diploid species. The section *Systylae* is made up of diploid species only, and he points out that this group is confined to the equatorial region. Diploid species and even diploid hybrids show regular reduction divisions, or very rarely a chromosome lags and is left in the cytoplasm. Other authors have found considerable proportions of bad pollen in some of these forms, and so he thinks that degeneration of the pollen grain is not always associated with irregular division.

In the tetraploid roses, regular division is general, but some species show poor affinity between univalents at diakinesis and sometimes the almost complete absence of bivalents. In forms showing exceptions to the regular meiotic divisions, there are other early signs of degeneration parallel to conditions found in tetraploid hybrids. The latter show always the characteristic irregularities found in the reduction divisions of polyploid forms.

The octoploid roses are represented, as yet, by a single species and a hybrid form. The species is circumpolar in its distribution in contrast to the circumequatorial distribution of the *Systylae* group of roses. The hybrid form has striking abnormalities in both the macro- and microsporangogenesis.

Triploid species have a reduction division that is parallel in every respect to that described by Rosenberg (19) for a triploid *Drosera* hybrid.

The Canina roses are, like the *Cinnamomea* roses, found mostly in the north temperate zone, and in the former group no species exhibits a chromosome condition that does not indicate a hybrid origin. The number and behavior of the chromosomes are such that he considers the Canina roses are  $F_1$  hybrids. He agrees with Miss Cole (4) that pollen sterility is the outcome of latent hybridity, and finds that pollen grains receiving bivalent and a very few univalent chromosomes are viable. A careful study of 40 divisions of the macrospore mother cells showed that the egg receives 7 chromosomes less than the somatic number of the species under consideration, although in a few cases some of the univalent chromosomes pass to the chalazal cells.

One group of hybrids he designates as *aneuploid*, for the bivalent and univalent chromosomes are not in multiples of 7, and even the somatic



number seems very irregular. All are characterized by a very slight tendency of the univalents to pair. He considers this group to represent the second or later filial generation of hybrids.

### CONCLUSIONS

The few scattered diploid *Crataegi* have a noteworthy range. Standish (21) has pointed out that the "stamping ground" for the probably heterozygous *Intricatae* and *Coccineae* is Massachusetts, southern Vermont, Connecticut, Rhode Island, New York, Pennsylvania, and Delaware. I find that the range of the diploid species is generally to the south, west, or north of the the above-outlined range, a few are found on the outskirts, and only one, *C. punctata*, which has a wide range, crosses this home of the heterozygous species. The Molles group, in which I have found the largest proportion of diploid species, has a distribution characteristically to the north and west.

The pollen-mother-cell development of diploid *Crataegi* is regular and indicates a genetic purity for species in this group.

The reduction division in triploid *Crataegi* differs very little from that described for triploid *Rubi* and for roses. The irregularities in chromosome-distribution are clearly those of a typical hybrid. Polyspory, an indication of much irregularity in pollen-formation, has been found in triploid and tetraploid species.

A more extreme abnormality of the pollen mother cells is the almost complete failure to form pollen. *Crataegus Smithii* is an example that shows degeneration within the anther similar to that described by Tischler (23). In the pollen grains of *Bryonia* hybrids the cytoplasm is much vacuolated, and the nucleus is thin except for the presence of chromatin material. Yasui (25) and Ljungdahl (13) have described divisions in *Papaver* hybrids very similar to those observed by the writer in this very sterile group of *Crataegus*. They found nuclei connected by a bridge of lagging chromosomes, and Yasui found that in some cells at about the anaphase of the homoeotypic division there appeared many vacuoles in the cytoplasm and an accompanying degeneration of the nucleus and cytoplasm took place. I have observed that the vacuolated condition of the pollen mother cells of these few sterile forms makes its appearance at or about the time of diakinesis. In most cases the cell degenerates at this early stage, but some few nuclei enter the heterotypic and even the homoeotypic division. The reduction of the chromosomes is carried out in much the same manner as in any hybrid species until the cells arrive at the final stages of degeneration, when the chromosomes clump together and form a compact mass. The division of this mass seems, at times, to be almost amitotic.

Hybrids may have a short life, never developing beyond a few cells. Others may mature into organisms vigorous vegetatively, but unable to produce flowers and fruit. Others are capable of producing spore mother

cells, but these fail to become tetrads. Some can carry out the reduction processes in a characteristic hybrid manner. In crosses between closely related species, pollen-formation shows few signs of irregularities, but the pollen shrivels. Finally, there are hybrids between species so closely related that practically no incompatibility exists between the sex cells, and they seem as normal as a typical homozygous species.

The two types of hybrids that a cytological study of the developing pollen mother cells reveals are those in which the tetrad is formed by a characteristic irregular division, and those in which the cells degenerate before a tetrad is formed. My study of *Crataegus* has revealed a number of this latter class. Their importance in this connection is the strong evidence they supply that they are hybrids, and, moreover, hybrids between species more distantly related than those producing the larger group of hybrids showing some irregularities in their pollen-formation. The latter group includes four fifths of the species I have examined.

Thus it seems that species of *Crataegus* have multiplied through hybridization. This conclusion, drawn from cytological investigations of many species, is substantiated by a study of the mature pollen. Guignard (11) in his study of hybrids noted that in the cross between *Mespilus germanica* and *Crataegus Oxyacantha* only one fourth of the pollen is viable. He found that not only is the pollen abortive in this hybrid, but that male organs as a whole are often poorly developed. Standish (21) studied a large number of species of *Crataegus* and found that only one fifth of these have normal pollen.

In conclusion, I draw attention to the mass of darkly staining material found in the cytoplasm in many species of *Crataegus*. Yasui (25), in her study of *Papaver* hybrids, described cytomixis. She says that nuclear material of the pollen mother cell, at the prophase, may pass into the cytoplasm of the neighboring pollen mother cell or cells through pits in the cell wall. I have found this dark-staining material most abundant in the prophase. I have seen what appeared to be an actual passing of this dark-staining material through the walls between a pollen mother cell and a tapetal cell. Similar observations have been made in the case of certain gymnosperms. Gates (9) shows, in his figure 41, bodies as large as chromosomes and taking the same stain scattered through the cytoplasm, which he thinks may be whole chromosomes or fragments of chromosomes, although he can not explain the cause of their expulsion. Digby (6), studying the meiotic nuclear divisions of *Galtonia candicans*, finds that chromatic bodies are given off from the nucleus during the presynaptic, synaptic, and hollow-spireme stages. An earlier paper (5) describes the origin of the chromatic bodies budding off from the nucleolus.

It is not intended to imply that the material in the cytoplasm taking much the stain of chromosomes is the same as that described by the preceding authors. The significance of this nucleoid material found so generally

in the polymorphic genus *Crataegus* is a problem beyond the scope of the present investigation.

#### SUMMARY

The *Crataegi* can be divided into three major classes:

1. Diploid species, in which I find the gametophytic chromosome number to be 16.
2. Triploid and tetraploid species that are able to develop their pollen mother cells to the tetrad stage.
3. Triploid and tetraploid species in which the pollen mother cells have thin and vacuolated cytoplasm and seldom develop to the tetrad stage.

The diploid *Crataegi* are characterized by normal pollen-formation.

The triploid and tetraploid species that are able to form pollen show irregularities in their chromosome-distribution that frequently lead to polycary and polyspory. These characters are clearly of the type associated with heterozygous species.

The triploid and tetraploid species that are unable to form pollen represent hybrids that are self-sterile and have resulted from crosses of distantly related species.

These polyploid forms show the following characteristics: pollen sterility, irregular chromosome-distribution, polycary, and polyspory—all features clearly associated with hybrid forms.

Finally, it seems reasonable to conclude that, in this polymorphic genus, multiplication of species has taken place by hybridization in their natural habitats.

In a brief paragraph, I wish to express my sincere thanks to Prof. C. S. Sargent of the Arnold Arboretum for the privilege of collecting the material necessary for this work and for suggestions he has made after reading this manuscript. This work has been carried on in the laboratories of plant morphology of Harvard University under the direction of Prof. E. C. Jeffrey, and to him I am much indebted for suggestions and assistance.

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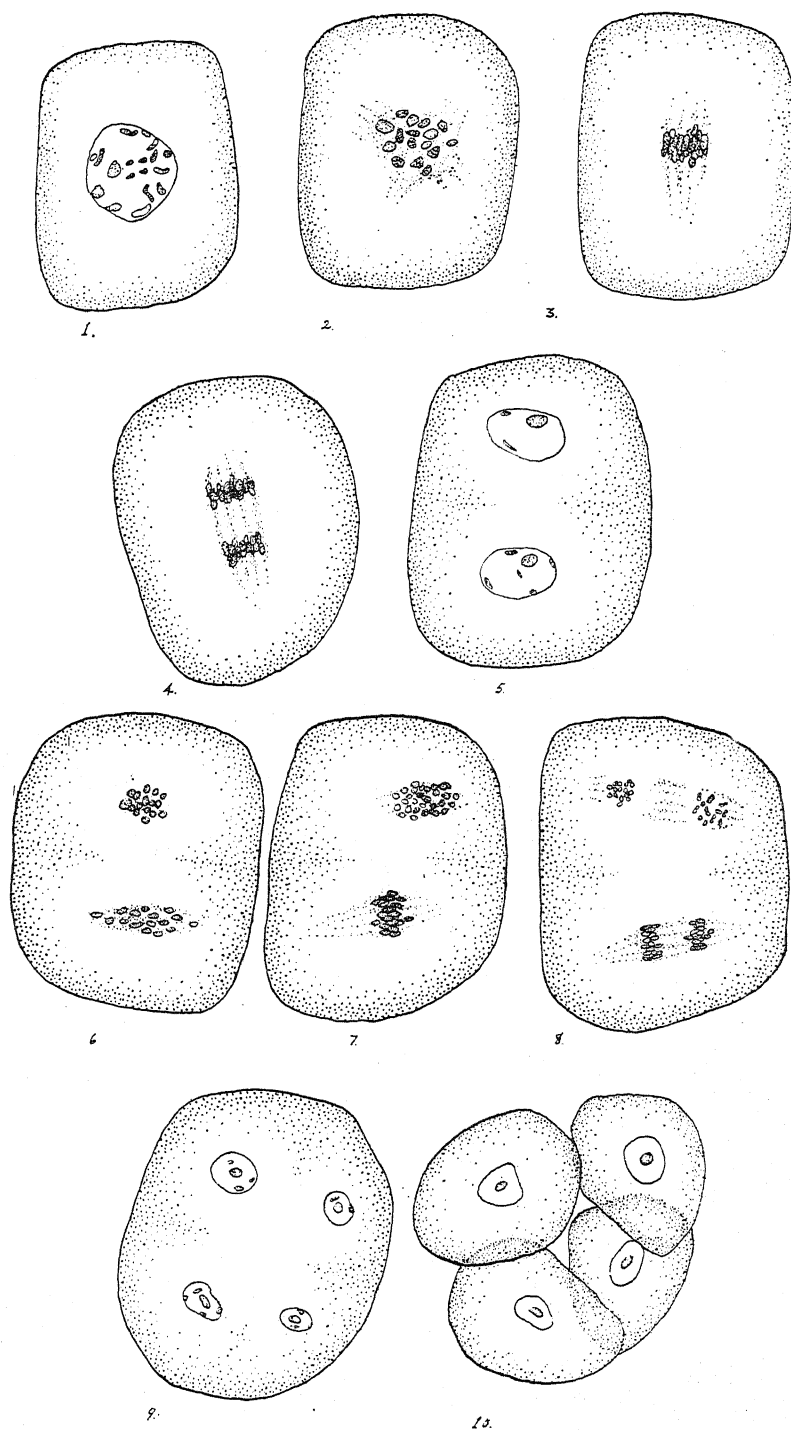
#### DESCRIPTION OF PLATES

The plates and text figures were kept in their true proportions by drawing at the height of the microscope stage with a camera lucida. I used a 1.5 mm. Zeiss apochromatic objective, a no. 12 compensation ocular, and the drawings were reduced one third in photographing.

#### PLATE XVI

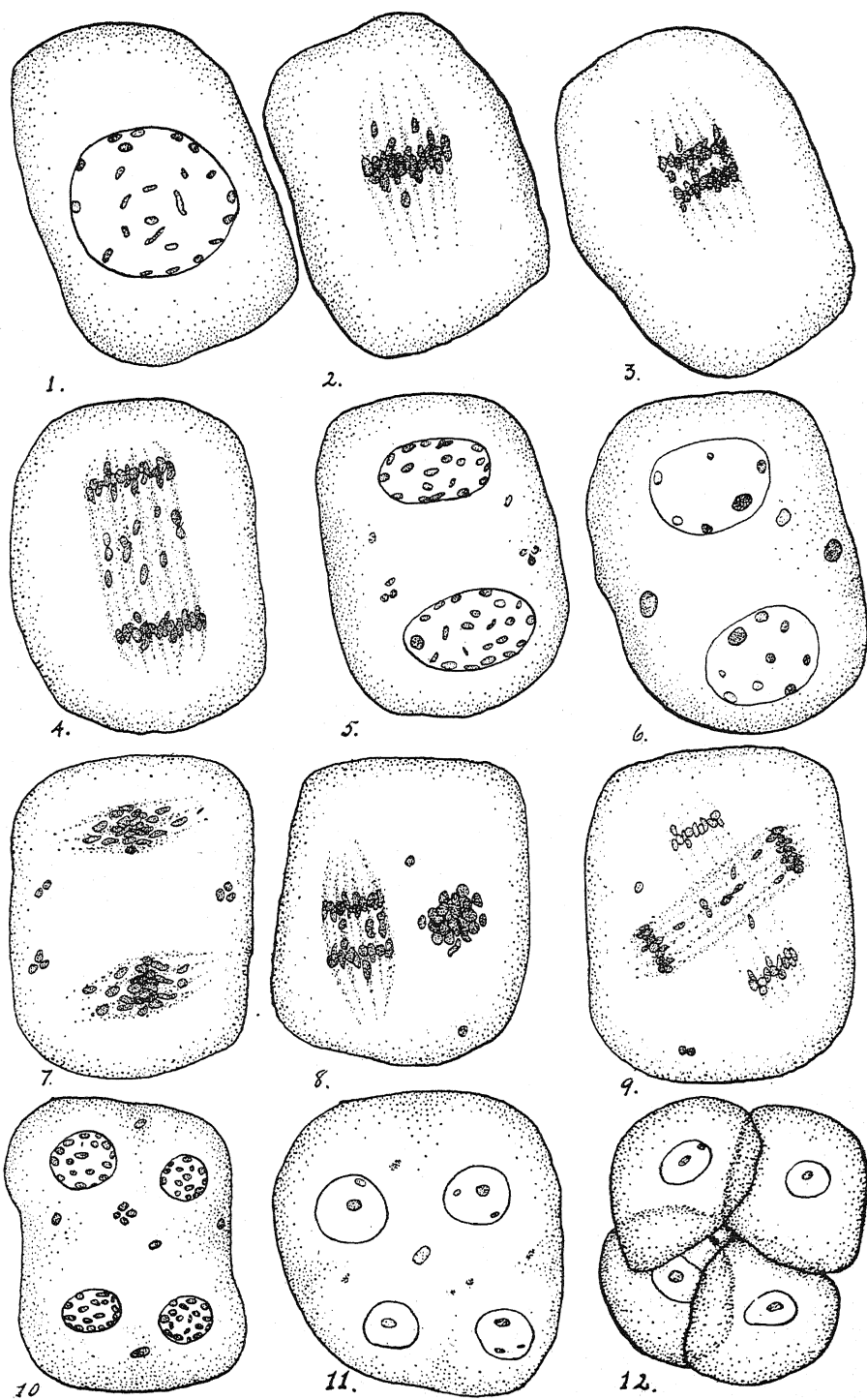
##### *Crataegus collina* Chapm.

- FIG. 1. Heterotypic prophase, a few chromosomes still unpaired.  
 FIG. 2. Heterotypic metaphase, showing 16 chromosomes on the multipolar spindle.  
 FIG. 3. Late heterotypic metaphase.



LONGLEY: CYTOLOGICAL STUDIES OF CRATAEGUS

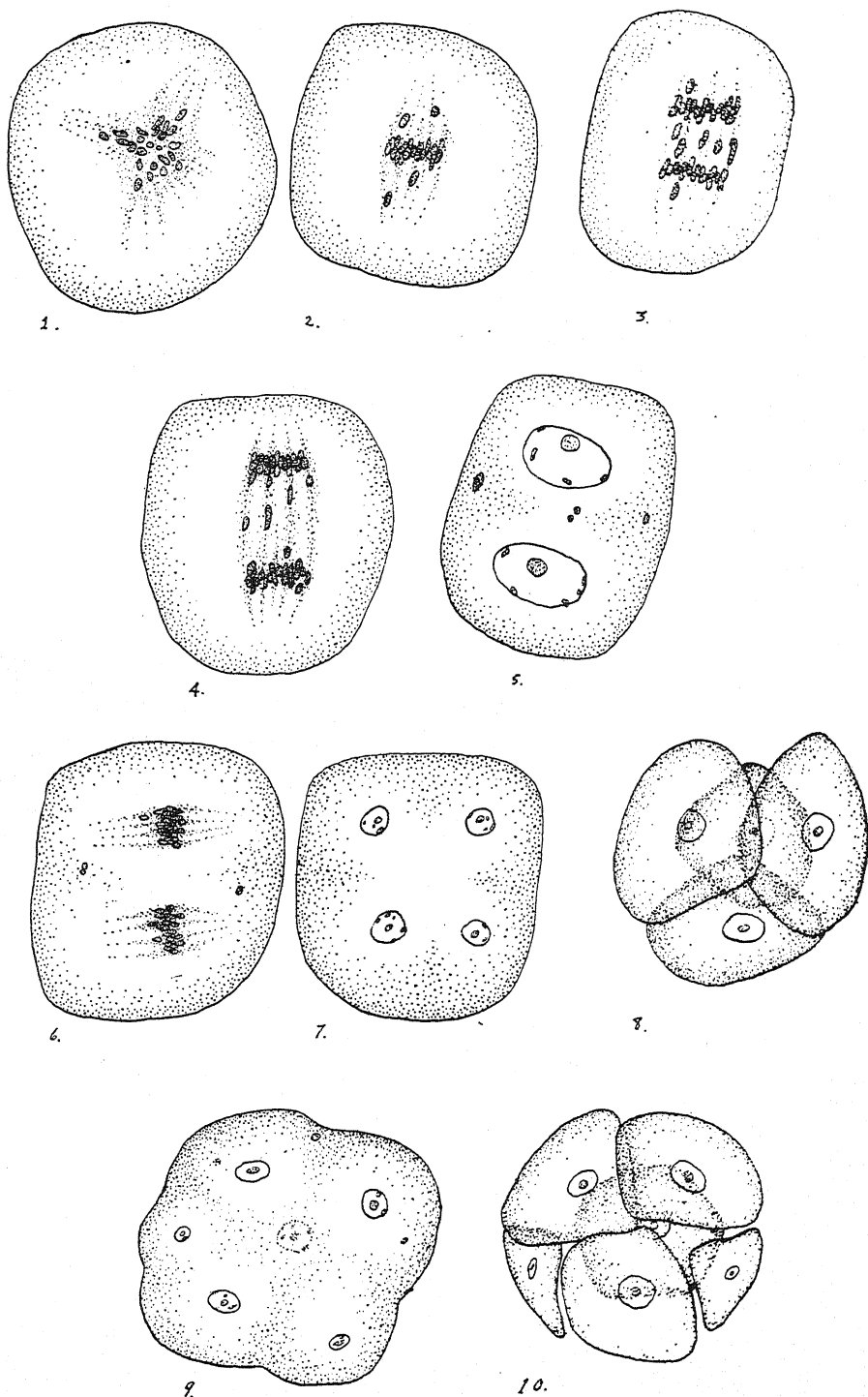




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LONGLEY: CYTOLOGICAL STUDIES OF CRATAEGUS



- FIG. 4. Heterotypic anaphase.
- FIG. 5. Interkinesis.
- FIG. 6. Homoeotypic metaphase.
- FIG. 7. Early homoeotypic anaphase.
- FIG. 8. Late homoeotypic anaphase.
- FIG. 9. Mother cell just previous to cell-plate-formation.
- FIG. 10. Tetrad.

PLATE XVII

*Crataegus pruinosa* C. Koch.

- FIG. 1. Diakinesis, showing 24 bivalent chromosomes.
- FIG. 2. Heterotypic metaphase.
- FIG. 3. Heterotypic anaphase.
- FIG. 4. Late heterotypic anaphase.
- FIG. 5. Late heterotypic telophase, showing chromosomes in the cytoplasm.
- FIG. 6. Interkinesis.
- FIG. 7. Homoeotypic metaphase.
- FIG. 8. Homoeotypic anaphase.
- FIG. 9. Late homoeotypic anaphase.
- FIG. 10. Early homoeotypic telophase, showing many chromosomes in the cytoplasm.
- FIG. 11. Mother cell, just previous to cell-plate-formation, showing chromatin material in the cytoplasm.
- FIG. 12. Tetrad.

PLATE XVIII

*Crataegus vicina* Sarg.

- FIG. 1. Early heterotypic metaphase, showing 24 bivalent chromosomes on the multipolar spindle.
- FIG. 2. Late heterotypic metaphase.
- FIG. 3. Heterotypic anaphase.
- FIG. 4. Late heterotypic anaphase.
- FIG. 5. Interkinesis, showing chromosomes in the cytoplasm.
- FIG. 6. Homoeotypic metaphase.
- FIG. 7. Mother cell, just previous to cell-plate-formation.
- FIG. 8. Tetrad.
- FIG. 9. Mother cell, just previous to cell-plate-formation, showing 4 major nuclei, 2 minor nuclei, and chromosomes in the cytoplasm.
- FIG. 10. Six pollen grains from one mother cell, two being dwarf.

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## A SILVICAL COMPARISON OF THE PACIFIC COAST AND ROCKY MOUNTAIN FORMS OF WESTERN YELLOW PINE

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Two botanical forms of western yellow pine have long been recognized: *Pinus ponderosa* Laws., typical of the Pacific Coast, and *Pinus ponderosa scopulorum* Engelm. of the Rocky Mountains. The diverse environmental conditions which characterize the habitats of these two forms also bring about silvical differences. These have been only partially recognized by foresters, and some interesting and marked dissimilarities have not as yet been clearly brought out. Some of these are of both scientific and practical importance, as is also the question whether biologic forms change readily in response to environmental conditions. In this paper it is proposed to emphasize some of the silvical differences between the Pacific Coast ("*ponderosa*") and Rocky Mountain ("*scopulorum*") forms and to point out their practical significance in forestation.

Within the vast range of western yellow pine the two forms are quite strictly segregated. The Pacific Coast form extends from southern California through Washington, Oregon, and southern British Columbia to central Idaho and western Montana, while the Rocky Mountain form occurs in eastern Montana, the Black Hills of Dakota, and southward through the Rocky Mountain region to northern Mexico.

In a study by Baker and Korstian (1), it was found that soil and climate have a profound influence upon the distribution of the two forms. The heavy clay soil of the pineless belt, often calcareous throughout, together with the characteristic deficiency and seasonal distribution of the precipitation, constitutes an effective ecological barrier. Each form is forced to its own side of the pineless belt. This belt is frequently two hundred miles or more in width.

Coupled with the botanical distinction above noted, this difference in silvical characteristics has resulted in considerable confusion as to the exact botanical status of the two forms. Taxonomists have disagreed as to their relative rank. The Rocky Mountain form has been elevated to specific rank (*Pinus scopulorum* (Engelm.) Lemmon); while still other authorities maintain that it is merely a climatic variant of *Pinus ponderosa*.

In advancing arguments in favor of the latter view, Sudworth (10) states that, in his opinion, much of the confusion has resulted from a study of herbarium specimens instead of the tree itself, as it is found growing under widely different climatic and edaphic conditions within its extensive

range. The two forms have often been distinguished botanically by the relative sizes of the leaves and cones. He maintains that, instead of attempting to separate the two forms botanically, as has been done on the basis of the relative sizes of leaves and cones, it is preferable to consider both as silvical forms of one variable species. Clearly, according to this view, the former characteristics are purely ecological; the size differences of leaves and cones, as well as the general vigor of the tree, are held to be due to the soil and climatic conditions under which the two forms grow. The similar cases of the Rocky Mountain and Pacific Coast forms of lodgepole pine (*Pinus contorta*) and Douglas fir (*Pseudotsuga taxifolia*) which grow under widely different climatic and edaphic conditions are cited as parallel examples.

For the purposes of the present discussion, however, the question of botanical nomenclature need not be pressed. Much more significant is the silvical difference between the two forms, which must be recognized in the rational silvicultural treatment of western yellow pine forests, whether it be in the regulation and natural regeneration of forests already established or in the starting of new ones. After more than 10 years' observation of the species in nearly every state in which it occurs naturally and in twelve different forest nurseries in the western United States, the writer had no difficulty in recognizing the two forms by the characteristics discussed in this paper, and it is believed that the differences are such that foresters normally can separate them just as readily.

#### DIFFERENCES IN APPEARANCE

When grown side by side in the same forest nursery, the two forms have consistently shown marked differences year after year. The one-year-old seedlings of the *ponderosa* form have leaves of a decided green or spinach-green color, occasionally bordered with bluish green, while those of the *scopulorum* form are blue-green with a decided tendency for the tips to be tinged with purple or bluish purple. The second year in the nursery, leaves of the latter form are of a rather light olive-green color while those of the *ponderosa* form are a pure green. Many of the two-year seedlings of the *scopulorum* form develop terminal buds, and by the end of the third year in the nursery practically all the transplants exhibit these. The *ponderosa* form, on the other hand, produces terminal buds at a greater age.<sup>1</sup> The terminal buds of the latter are a reddish brown while those of the *scopulorum* form are a brownish gray. The bark of three-year-old transplants of the two forms shows a corresponding contrast in color.

<sup>1</sup> This has not been wholly borne out by the experience of G. A. Pearson, Director of the Fort Valley Forest Experiment Station, who found that in northern Arizona the *scopulorum* form does not develop terminal buds until about four years old and occasionally later. On the other hand, three-year-old stock of this form from Colorado almost invariably has well developed terminal buds.

In the juvenile stage, as great or greater differences between the silvical characteristics, especially color, size, and general appearance, of these two forms of western yellow pine were exhibited in the Cottonwood Nursery than between the *scopulorum* form and lodgepole pine, both collected in the Wasatch National Forest within 30 miles of the nursery. As the trees of the two forms grow older the juvenile characteristics largely disappear, and the distinguishing characters are confined mainly to the structure, size, and color of the leaves, size and color of cones, and the color of the pistillate flowers. The leaves of the *ponderosa* form are 5 to 13 inches long, while those of the *scopulorum* form are shorter—3 to 6 inches. The cones of the latter are light yellowish brown on the exposed surfaces of the scales, reddish brown on the unexposed parts, and 2 to 4 inches long, while the *ponderosa* cones are purplish and 3 to 8 inches long, generally longer than the *scopulorum* cones. The pistillate flowers of the *scopulorum* form are purple, those of the *ponderosa* dark red.

#### DIFFERENCES IN BEHAVIOR

The duration of the germination period and the size of the seed are two features in which a significant contrast between the two forms has been noted. A long germination period seems to be a characteristic of the *ponderosa* seed,<sup>2</sup> which is also considerably larger than that of *scopulorum*. A difference which possibly has a bearing upon the period of germination is one which has been observed in the size of the seeds of the two forms. A number of representative samples of seed of the *ponderosa* form collected in the Boise, Payette, Idaho, and Salmon National Forests in central Idaho ranged from 8,380 to 11,120 seeds per pound, with an average of 9,750 for the region. Samples of seed of the *scopulorum* form collected in the Ashley, LaSal, Sevier, and Wasatch National Forests in Utah ranged from 14,400 to 21,300 with an average of 17,280 seeds. Toumey (11) has pointed out that there is a striking correlation between the size of the seed and germination when the available moisture or growth water in the surface soil is deficient at the time of germination and immediately following. The larger size of the *ponderosa* seed is attributed to the need for a greater supply of reserve food material in the seed in order that the seedling may rapidly develop a deep root system sufficient to tide it over a protracted dry season.

In early rate of growth, also, there is evidence of a wide difference between the two forms. At the Cottonwood Nursery in central Utah, where the two were raised extensively for a number of years, nursery stock of the *ponderosa* form was always larger and more vigorous, producing a better root system, composed of more fibrous laterals, as well as a thicker stem. Two-year-old seedlings of the *ponderosa* form had tops averaging 3.5 inches

<sup>2</sup> The *ponderosa* form has shown a strong tendency toward delayed germination when sown in northern Arizona and central California, while in central Utah it requires a germination period not quite twice as long as that required by the *scopulorum* form.



tall and weighing 3.85 grams each, stems 0.14 inch in diameter at the root collar, and roots 11.6 inches long weighing 1.30 grams each. *Scopulorum* seedlings of the same age grown under similar conditions had tops averaging 2.2 inches tall, averaging 1.49 grams in weight, stems 0.11 inch in diameter at the root collar, and roots 9.1 inches long with an average weight of 0.41 gram.

Notwithstanding its superior development, the *ponderosa* form when transplanted into the field succumbs more quickly to drought. In northern Arizona this form is also quite susceptible to frost, while the native *scopulorum* is frost-resistant.

A special study of snow molding (6) has showed that seedlings of the *ponderosa* form grown from seed collected in central Idaho are much more susceptible to the snow-molding fungi than those of the *scopulorum* form grown from seed collected in the vicinity of the Cottonwood Nursery.

#### CHEMICAL DIFFERENCES

The oleoresin of the two forms, when analyzed chemically, was found by Schorger (9) to be distinctly different, justifying their separation on this basis if for no other reason. The *ponderosa* form was reported to contain about 65 percent of beta-pinene with a very small amount of alpha-pinene, while the *scopulorum* form contained about 65 percent of the latter terpene. The oils of the former are laevo-rotary while those of the latter are dextro-rotary.

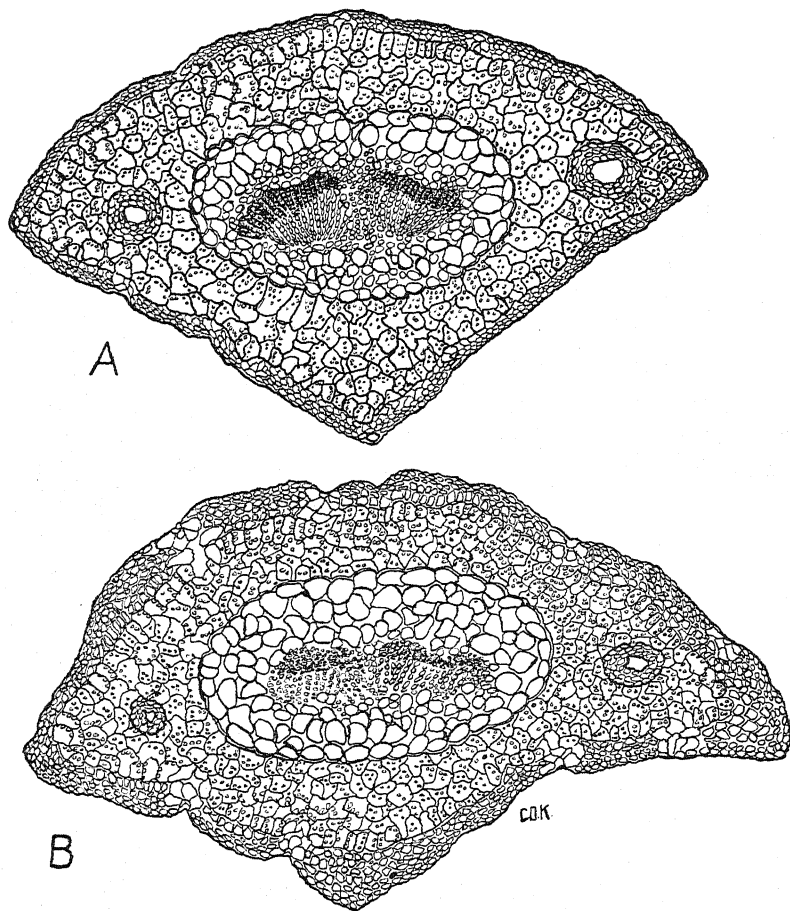
In the case of the seed oils of the two forms, less striking results were obtained by Ritter (8), who determined the physical and chemical constants commonly used in the examination of the fixed vegetable oils. The examination of the oil expressed from seed of the two forms showed higher numerical values for *scopulorum* than for *ponderosa*, but the differences were too small to be wholly satisfactory as an aid in differentiating one from the other.

That the volatile leaf oils of the two forms differ strikingly in chemical composition was suggested by an observation made by the writer (7) in the course of some studies on cell-sap density. The leaves and freshly expressed cell sap of the *ponderosa* form have a pleasant, mild, resinous fragrance, reminding one of the pine woods after a rain. On the other hand, the expressed sap of *scopulorum* leaves has a much stronger, but less pleasant, terebinthine odor. When this difference was found to be characteristic, the writer used it with confidence as a convenient means of distinguishing between the two forms. It is possible that chemical analyses would reveal a definite reason for this difference in odor.

#### DIFFERENCES IN LEAF STRUCTURE

The fundamental differences between the two forms are further emphasized by comparing the microscopic structure of typical leaves of both

grown from seed under the same habitat conditions (text fig. 1). The layer of thick-walled hypodermal cells which is continuous all around the leaf in both forms is considerably thicker in the *scopulorum*. The leaves of the latter have the more compact structure, are thicker, the cells lie closer together, and the stomata appear to be more deeply depressed. All



TEXT FIG. 1. Camera-lucida drawings of cross sections of leaves of 7-year-old western yellow pine saplings grown side by side in the same forest plantation: A, *Pinus ponderosa* Laws.; B, *Pinus ponderosa scopulorum* Engelm.

of these anatomical modifications tend to retard evaporation, and are therefore characteristic of the more xerophytic conditions to which the *scopulorum* form has long been subjected in nature. Since the leaves are the organs of photosynthesis and transpiration—physiological activities profoundly influenced by environmental conditions—it is logical to find that the adaptational response of the tree to its habitat is portrayed by the structure of

the leaf. These two forms reflect in the anatomy of their leaves the great divergence in environmental conditions between the Rocky Mountain and Pacific Coast regions. Henry and Flood (5) have shown a striking analogy in the leaf structure, as well as in the size of cones and in the presence of different oils, between the Pacific Coast and Rocky Mountain forms of Douglas fir in relation to their distribution and the environmental conditions to which they are subjected.

The experiments of Bonnier (2) are of interest in this connection. Plants previously grown under similar conditions were set at various altitudes in the Alps and Pyrenees. In general, all lowland plants belonging to species naturally able to tolerate the differences in altitude develop well under alpine conditions. At the end of 30 years in the high altitudes, nearly all lowland plants have assumed the habits and anatomical structure identical with those of plants of the same species already growing at the same altitudes. Complete adaptation of this sort is accomplished in 8 to 10 years by some species, whereas others require more than 25 years. Brenner (3) concludes from his experiments and anatomical studies on leaves that modifications caused by the environment are hereditary and may lead to the development of new species.

#### SUMMARY

The above discussion indicates that the two forms of western yellow pine possess definite inherent silvical characteristics which may be transmitted hereditarily from one generation to another. Although taxonomy is based largely upon morphological characters, it should not completely overlook the influence of the climatic and edaphic factors of the habitat. In other words, climatic forms, or varieties possessing different silvical characteristics, should be recognized just as freely as those based on morphological characters. Cieslar (4) has emphasized the importance of climatic varieties of forest trees in the practice of silviculture. Experience abroad has shown that, in plantations grown from exotic seed, undesirable qualities that have lain dormant may appear after 20 or 30 years. With this in mind, the only safe policy in silvicultural practice and planting is to take due cognizance of the silvical differences between *Pinus ponderosa* and *Pinus ponderosa scopulorum*. In planting either form, the seed used should be locally collected from sites similar to the ones to be reforested. Introduction of either form on lands outside of its natural range should be on an experimental basis only, until long-time experiments have shown conclusively that it is adapted to the particular site under consideration.

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# THE TRANSFORMATIONS AND COURSE OF DEVELOPMENT OF GERMINATING MAIZE

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## INTRODUCTION

One prime feature of plant study, both from the scientific and from the crop-production viewpoint, is the determination of the modifications in plant development with the variation of the factors of growth. Klebs (1903) has been a leading contributor to the developments in this field by his studies of the modifications of the life histories of plants under varied environment. But, although a change in the development of a plant is a response to a change in the environment, this response is the immediate result of internal processes. Kraus and Kraybill (1918) have partially developed this viewpoint and have shown a relation of internal composition to plant development; they studied the relation of the balance of two of the classes of substances (carbohydrates and nitrates) used by the plant in building its tissues, on the fruitfulness or vegetativeness of the tomato plant.

Blackman (1919, 1920) and Kidd and West (1918-19, 1920) have recently indicated that the growth of a plant directly produced from seed is a function of the early development of the seedling. Kidd and West have designated this relation as "predetermination" of the seed or seedling. From an extensive review of the literature on the subject, they found abundant evidence that crops are directly influenced by the physiological condition of the seed and of the seedling, independently of their hereditary qualities. Gassner (1918) has given striking evidence of the influence of external factors during the time of germination of the seed on the future course of development of the plant, by showing that winter cereals will develop to maturity in a single season if subjected to a low temperature during the period of germination of the seed.

Although the volume of literature on seed-germination is large, knowledge of the transformations which occur in the seed and seedling during germination is far from complete. Also, the data on the influence of specific external factors on germination are confusing, for it has been found that a particular type of response may accompany any one of several external factors. In other words, there is need of a better understanding of the metabolism of the seedling as it develops in different environments. A satisfactory control of the process of germination must be based on a knowledge of the composition of the seed, and of the changes in the substances

which make it up during the course of its development under varying conditions.

In the present work, an attempt has been made to follow some of the histological and chemical changes that take place as germination progresses under an arbitrarily chosen, standard set of environmental conditions. Naturally, the next step should be to follow these internal transformations as the environment is varied, one factor at a time. Such a study should reveal the dependence of the various processes of development on the separate external factors. From this viewpoint, a preliminary survey has been made of the modified development of seedlings.

It is clear that the desired information concerning the internal transformations can not be secured by an analysis of the entire seed or seedling, since even a cursory examination will show the marked differences in composition of the different parts of the seedling. In order to correlate, for example, the growth of the root with chemical changes, it is necessary to find the changes occurring within the root itself as well as elsewhere. By microchemical methods, that are now well developed, it is possible to obtain a very close approximation to the actual amounts of many substances as they exist in a cell or a tissue. But, although the results of a microchemical study are invaluable, it is best to check and extend this information by quantitative analyses of seeds or seedlings at the more critical stages in germination.

#### HISTORICAL

The study of seed-germination has been, for a long time, a favorite method for studying the physiology of the plant. Although there was much early work on seed-germination and especially on the germination of the grains, Sachs was the first to make a striking contribution to this field by his attempt to follow the chemical changes and movements of the reserve materials in the seed and their ultimate utilization in the development of the seedling. This work depended to a large extent on the development by Sachs (1862 *a*) of suitable microchemical methods for distinguishing the various organic materials that might be present.

He found (1859) that in the germination of several oily seeds there was an early appearance of starch in the hypocotyl. With the beginning of growth of the various organs, sugar appeared in them. In a detailed microscopic and chemical study of the germination of *Phaseolus multiflorus* (1862 *a*), he followed the transfer of carbohydrates and proteins from the cotyledons to the tissues of the seedling. The carbohydrates were stored in the cortex and pith of the seedling, while the proteins accumulated only at the growing points.

Sachs' discussion of the germination of grasses (1862 *b*) brought out the main outstanding facts concerning what takes place in the early growth of this group of plants. The greater part of the work was with maize, although a comparison was made with other grains. He recognized the epithelial

layer of the scutellum as an absorbing and secreting organ. Starch was *never* found in the epithelial layer. The walls of the parenchymatous cells of the scutellum have netted thickenings. These cells contain nuclei, protein granules, and oil drops; in maize there are small, round starch grains, but these are not found in wheat. The first stages of germination are not accompanied by the appearance of sugar or dextrin in the endosperm. During the early development of the seedling, the material already present in the embryo apparently is used. When the radicle of maize is about three centimeters long, the starch of the endosperm first shows corrosion, and sugar and also dissolved proteins are found in the endosperm cells. These changes in the endosperm were first detected in the cells next the scutellum; the digestion then proceeds slowly to the other parts of the endosperm. There appear in the parenchymatous tissues of the embryo fine starch grains, which are replaced by sugar as the cells of these tissues enlarge, and finally the sugar disappears, having been used in building the new cell walls. In older seedlings, starch is found only in the starch sheath. Neither sugar nor starch is found in the epithelium at any time during germination. Although transitory starch grains are seen in the scutellum, this tissue also remains free of sugar or dextrin. His only explanation of the method of passage of carbohydrates through the scutellum assumed that, after having passed through a wall, sugar is immediately changed to starch, that small amounts of this starch are in turn changed to sugar which passes through the next wall and is there again immediately changed to starch, and so on. The relative growth of embryos deprived of reserve food at various stages of germination indicates that the greatest influence of the endosperm material is during the first few days of germination.

Gris (1864) did not agree with Sachs' idea of the successive solution and reformation of starch in the cells of the scutellum during the transfer of food from the endosperm to the growing embryo. He did not explain in any way the transfer of carbohydrates, but simply said that "the scutellum is the principal organ of absorption of the embryo, a sort of neutral intermediary between a tissue which it absorbs, the endosperm, and a tissue which it develops, the embryo proper."

Detmer (1880), in his comprehensive book on seed-germination, gave a detailed account of previous work on the germination of grasses. Also, he presented some original data from a chemical study of the changes in dextrose and starch in entire seedlings of *Zea mays*.

Brown and Morris (1890) investigated the chemical and histological transformations during the germination of barley. The first visible change observed during the germination is the elongation of the radicle, accompanying which, starch appears abundantly in the coleorhiza end, in the periblem of the radicle, and in the cells of the plumule, but not in the scutellum. When the radicle breaks through the coleorhiza, starch first appears just underneath the epithelium, and then its progress follows inward



through the scutellum. At the time of this first appearance of starch in the scutellum (after 24-36 hours of germination), there occurs in the endosperm a softening and partial dissolution of the cellulose walls of the layer of empty cells lying next the epithelium. Then the walls of the starch-containing cells break down. The starch grains themselves are never attacked until after the breaking down of the walls. The cell walls appear swollen and stratified before solution; the middle lamella has greater resistance. As the tissue of the endosperm is digested, it is compressed by the advancing embryo. The starch of the endosperm is converted to maltose, but on entering the scutellum it is quickly changed to sucrose. Table I, from Brown and Morris' paper, shows the increases in the various sugars during germina-

TABLE I

	Barley after Steeping in Water for 48 Hours		Barley after Germinating for 10 Days	
	Embryos	Endosperms	Embryos	Endosperms
	(percent)	(percent)	(percent)	(percent)
Cane sugar . . . . .	5.4	0.3	24.2	2.2
Invert sugar . . . . .	1.8	0.2	1.2	2.2
Maltose . . . . .	—	—	—	4.5
Total . . . . .	7.2	0.5	25.4	8.9

tion. In this connection, it should be mentioned that Kjeldahl (1882) and O'Sullivan (1886), both having worked with the entire grain of barley, found a similar large increase in sucrose during malting, but also found a marked increase in dextrose.

Brown and Morris made a careful study of the enzymes concerned in germination and of the tissues involved in their elaboration. Because of the ease with which it was possible to grow the excised embryos on endosperms of other seeds or on most starches, except those from bean and potato, they concluded that the embryo is the source of the amylo-hydrolytic enzyme. They further localized the formation of this enzyme in the epithelial layer of the scutellum, but thought that nitrogenous substances entering from the endosperm constitute the "mother substance" from which the epithelium forms the diastase. They were able to isolate a separate cyto-hydrolytic enzyme, also secreted by the absorptive epithelium. Since the secretion of digestive enzymes is localized in the embryo, and there is no observable indication of vitality in the endosperm, they "distinctly favor the view that the endosperm of the grasses is a dead store-house of reserve material. Under these circumstances the life of the embryo during the germinating period is strictly speaking not parasitic but *saprophytic*."

Haberlandt (1890), just previous to the work of Brown and Morris, had suggested that the cells of the aleurone layer of the endosperm are an active

agent in diastase-production. Brown and Morris considered this point very carefully in an addendum, and concluded that "the aleurone cells belong solely to the reserve system of the seed."

The work of Brown and Morris on the transformations during germination should have been a stimulus to more detailed work on the subject, but most of the work since then on the germination of the seeds of grasses has been along the line of their incidental suggestion that the grass endosperm is "dead."

Hansteen (1894), by providing for the removal of the products of digestion by a plaster-of-paris block immersed in water, found the endosperm with embryo removed capable of self-digestion. Linz (1896), by the use of quantitative methods for diastase determination, found that the scutellum of maize contains diastase, but that there is no evidence of its secretion. Grüss (1897), from work on maize, concluded that "seedlings, from which the endosperm has been removed, are able, without the presence of bacteria, to nourish themselves on a starch paste, which is thereby made into sugar." Puriewitsch (1898) obtained practically the same results as Hansteen. Brown and Escombe (1898) found evidence of cyto-hydrolytic and probably some starch-digesting power of the aleurone layer. Miss Bruschi (1908) concluded that there is a varying proportion of living substance in the endosperms of maize, barley, wheat, and rye.

Le Clerc and Breazeale (1911) made a study of the changes in both inorganic and organic constituents of wheat seedlings under different conditions of nutrition in water cultures in the light. No reducing sugar was found in the original seed, but a small amount of hydrolyzable sugar was present. In the partly depleted endosperm there was a gradual decrease of hydrolyzable sugars and a marked increase in reducing sugar up to the fifth day. In the top and roots there was a very rapid accumulation of reducing sugars during the first five days. The hydrolyzable sugar of the entire seedling never increased, but decreased during the last few days.

Mann and Harlan (1915) made a careful study of the barley grain with reference to its ability to produce enzymes for the brewing industry. During germination, they found an early appearance of starch in the embryo, which is free from starch at maturity. They concluded that the epithelial layer of the scutellum is the effective source of starch- and cellulose-dissolving enzymes.

In connection with other work, some data have been accumulated on the chemistry of the resting seeds of the grass family. Stone (1896) found the dry corn grain to contain less than one half percent of sucrose, no invert sugar, a trace of dextrin, over five percent of pentosans, and two percent of crude fiber; at that time he found only 42.5 percent starch, but later (1897) he stated the starch to be about 65 percent.

Hopkins (1898) made a study of the chemistry of the corn kernel from which the following average percentage composition was obtained: ash

1.4; protein 8.35-13.8; fat 3.95-6.0; carbohydrates 79-85.8. The carbohydrates are largely starch, but there are 5-6 percent pentosans, 2 percent crude fiber, and a trace of sugar. The oil was found to contain over one percent of cholesterol and  $1\frac{1}{2}$  percent of lecithin. The germ contains twice the mineral matter and from three to four times as much oil as the remainder of the kernel.

Hopkins, Smith, and East (1903) made a more detailed analysis of the composition of the different parts of the corn kernel. Table 2 is compiled from their data.

TABLE 2. *Approximate Composition of the Parts of a Corn Kernel*

Part	Percent of Whole	Protein (percent)	Oil (percent)	Ash (percent)	Carbohydrates (percent)
Whole corn.....	100.0	10.9	4.33	1.55	83.17
Germ.....	11.5	19.8	34.8	9.9	35.5
Endosperm.....	88.5	9.7	0.83	0.42	89.0
Composition of germ as percent of whole corn.....	11.5	2.3	4.0	1.14	4.1

The horny gluten (aleurone) was found to contain from 19 to 24 percent of protein and from 4 to 7 percent of oil.

Winterstein and Wünsche (1915) analyzed commercial maize germs and found 53-56 percent of crude fat, 1.37 percent of ash, 2.0 percent of total nitrogen, 1.8 percent of protein nitrogen, and only traces of reducing sugar or sucrose. Boiling with concentrated  $H_2SO_4$  gave small amounts of a pentose. Sitosterin, lecithin, and phytin were present. They found traces of water-soluble protein and globulin, but most of the protein was soluble in alkali.

Miss Choate (1921) followed the chemical changes which occurred in wheat during germination under an arbitrarily chosen, standard set of conditions. Microchemically she found that the principal storage carbohydrate of the wheat grain is starch in the endosperm; a small amount of sucrose was also found. The embryo is free of both starch and sugar. The first apparent chemical change (after 10 hours) is the presence of starch in the root cap and of dextrin in the scutellum and coleorhiza. A few hours later, reducing sugar is found in the coleorhiza, and soon afterward this sugar is present in all parts of the seedling. Peroxidase and catalase are present in all parts of the grain before and after germination. A quantitative study of the catalase showed an increase in its activity during germination corresponding to an increase in respiration. Analysis showed the presence of amino-acids in the ungerminated grain (0.024 percent) and their increase during germination.

#### MATERIALS AND METHODS

The greater part of the corn used in this work was of the Ninety Day Yellow Dent variety grown at Bloomington, Illinois, in 1919, and obtained

through the kindness of Mr. J. R. Holbert of the Office of Cereal Investigations of the U. S. Department of Agriculture. The ears used were of a disease-resistant strain and were grown from a single mother ear. The corn had been stored in an airy, dark, dry room with a fairly constant temperature near 22° C.

Unless otherwise mentioned, the corn was germinated in covered dishes, on filter paper kept moist with distilled water, in a dark room the temperature of which was fairly constant; the greatest range of variation being from 21° to 24° C. Conductivity water, showing no evidence of toxicity, was used to moisten the filter paper.

The progress of the histological and chemical changes during germination was studied from free-hand sections. The microchemical methods used were those described by Eckerson, Molisch (1913), and Tunmann (1913). All tests were repeated as many times as seemed necessary in order to leave no doubt of the correctness of the findings.

The results from the microchemical study were checked at three different stages of germination by quantitative determinations of the more important constituents. The details of the methods used are given in connection with the presentation of the quantitative results.

#### STUDIES OF DEVELOPMENT

##### Anatomical and Histological

The difference in embryo-structure between the Gramineae and other monocotyledons has attracted much attention and has caused considerable discussion as to the homology and the proper terminology of the various parts. It seems necessary at the start to have clearly in mind the relations of the various parts of the embryo, and to adopt some definite terminology.

In maize, the main questions to be considered are those involving the homology of the scutellum, the coleoptile, and the internodal tissue below the plumule and above the place of attachment of the scutellum, with the parts of other embryos. Sargent and Arber (1915) have reviewed the earlier literature and have discussed the question in reference to the entire family of the Gramineae. From a study of the vascular elements and of the process of germination, they concluded that, in the grass embryo, "the *scutellum* is the sucking apex of the cotyledon" and the coleoptile is the cotyledonary sheath. "The *mesocotyl* is the fusion of the cotyledonary stalk with the hypocotyl." They found the anatomy of the embryo and seedling of maize to be in harmony with this conception of the grass embryo.

Worsdell (1916), from a review of the literature and from observations on *Zea mais*, concluded that the scutellum is the lamina of the cotyledon, that its sheath is present only during the early embryonic development, that the coleoptile corresponds to the ligule, and that the epiblast, when present, corresponds to the auricles of the foliage leaf. The stages of em-

bryonic development were interpreted as showing that the cotyledon is a terminal structure. The mesocotyl was looked upon as an elongated first node.

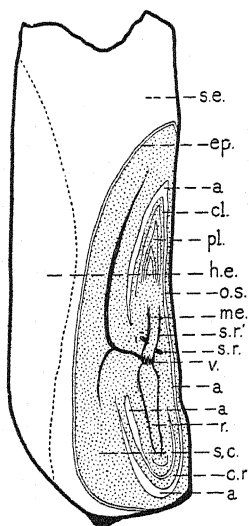
The great range of viewpoints is illustrated by these more recent papers. But none of these attempts to homologize the parts of the grass embryo with well understood seed-parts helps to interpret the structures found in maize seedlings. No attempt has been made by the writer to study the exact homology of parts or to verify the evidence of Sargent and Arber, but their conception has the distinct advantage of explaining the lack in maize of any stem-like structure below the region of attachment of the scutellum, as well as the similarity of the mesocotyl to a hypocotyl and the lack of symmetry of the bundles of the mesocotyl. So it has, for convenience, been assumed in this work that the part of the embryo below the attachment of the scutellum and enclosed by the coleorhiza is the true radicle; that the region between the attachment of the scutellum and the plumule may best be

called the mesocotyl, and is, in function at least, in part the hypocotyl; and that the coleorhiza and possibly also the coleoptile are outgrowths of the cortical tissue of the mesocotyl.

The general structure of the dry grain of maize is shown in text figures 1 and 3. In the dry grain, the surface of the embryo is shrunken considerably below the level of the endosperm, as is shown especially in a cross section of the fruit (text fig. 3). In the embryo, the tissues of the radicle, of the coleorhiza, and of the plumule are shrunken away from one another, leaving distinct open spaces within the grain (*a*, text figs. 1 and 3). Even the protoplasts of the individual cells of the embryo are shrunken away from their walls.

The first change, after placing the grain under conditions suitable for germination, is the imbibition of water by all the cells until the cells are turgid. The open spaces of the dry grain become entirely filled, and the face of the germ becomes level with the surface of the endosperm. From ten to twelve hours are required for this to take place, although the time varies greatly. There seems to be no actual enlargement of the cell walls, nor can chemical change be detected.

About twenty hours after imbibition has begun, there can be observed an enlargement of the cells of the coleorhiza, which breaks the fruit coat and extends about two millimeters beyond the surface of the grain. The radicle fills the extending sheath, but there is no evidence of cell division in either structure. A few hours later, the elongating radicle breaks through



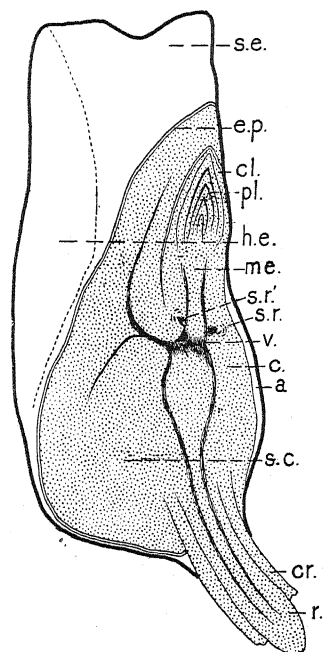
TEXT FIG. 1. Longitudinal section of dry grain.

the sheath which does not develop further. Cell division may be first observed in the embryonic region of the root at about the time that it breaks through the coleorhiza.

At this same time, there begins an enlargement of the cells of the cortical tissue on the face of the embryo, between the radicle and the plumule, which merges with the base of the coleorhiza and with the scutellum (*c*, text fig. 2). The cells of this tissue on the exposed face of the embryo enlarge to a remarkable extent, but there is never any sign that cell division takes place. The nuclei of these cells become very much enlarged and very distinct. After about 48 hours, when the radicle is from 1 to 2 cm. long, the mesocotyl begins to elongate and pushes the plumule through the fruit coat. The early development of the mesocotyl is always much slower than that of the radicle.

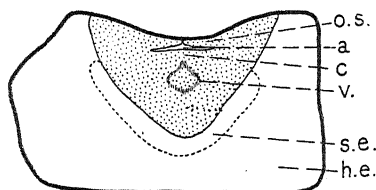
Accompanying the elongation of the mesocotyl, there is a noticeable elongation of the secondary root initials which are already present in the dormant seed (*s. r.*, text fig. 1). First to develop are two initials, one on either side of the median dorsal line of the base of the mesocotyl (*s. r'*, text fig. 2), which elongate into the space between the inner surface of the scutellum and the plumule. In some cases there is another initial on either side of these, which, however, is slower in developing. Another secondary root initial on the face of the seedling, opposite the point of attachment of the scutellum (*s. r.*, text fig. 2), pushes its way through the swelling cortical tissue at about this same time.

Shortly after the first elongation of the radicle there is apparent a noticeable elongation of the cells of the epithelium of the scutellum and a swelling of the ends of those cells which are most elongated. Also, the surface is increased by the development of fissures in the epithelial surface. The structure of the scutellum and epithelium has been carefully described by Sargent and Robertson (1905). The face of the scutellum does not seem to develop evenly, but conspicuous bulges appear on the face where the epithelium seems



TEXT FIG. 2. Longitudinal section of grain after about forty-eight hours' germination. *h. e.*, horny endosperm; *s. e.*, starchy endosperm; *sc.*, scutellum; *ep.*, epithelium; *cr.*, coleorhiza; *r.*, radicle; *s. r.* and *s. r'*, initials of secondary radicles; *v.*, vascular tissue; *cl.*, coleoptile; *pl.*, plumule; *me.*, mesocotyl; *c.*, cortical tissue which greatly enlarges in germination; *a.*, air spaces between tissues in the grain; *s.c.*, scutellum overlapping face of embryo.  $\times 5$ .

most active, especially toward the lower part opposite the point of attachment of the scutellum to the seedling (text fig. 2).

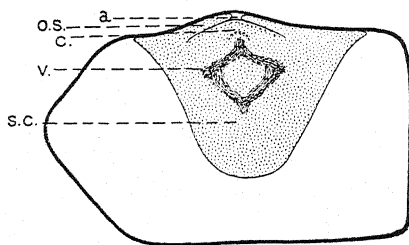


TEXT FIG. 3. Transverse section of dry grain in the region of the vascular plate.

In later stages of germination, as the endosperm undergoes depletion, the scutellum advances by an enlargement of its individual cells to much more than their original size. This enlargement and advancement of the scutellum into the endosperm region may be observed by a comparison of text figures 1 and 2. The procambial strands of both the scutellum and the growing parts of the embryo gradually develop and differentiate into vascular tissue.

In the endosperm, both the contents and the walls of the cells with which the epithelium of the advancing scutellum comes into contact are digested and absorbed.

The growth of the corn seedling may be looked upon as divided into three stages. The first is the imbibition of water by the seed. This is accompanied by no marked enlargement of the cell walls. The amount of water taken up varies greatly for the different structures. The imbibed endosperm contains only about 25 percent of water, but there is approximately 60 percent in the embryo. Babcock (1912) attributed the greater imbibition by the embryo entirely to its more porous structure. The writer considers, however, that its chemical composition is the reason for the difference.



TEXT FIG. 4. Transverse section at the same point as figure 3, but after forty-eight hours' germination.  $\times 5$ .

The second stage of growth is accompanied by an enlargement of the cells and by a stretching of the cell walls, which result in a decided increase in the size of those tissues that are growing. This growth takes place very



unequally in different parts and may be considered the beginning of the growth of the embryo. Babcock considers that much of the water contributing to the turgidity of the cells at this stage is "metabolic water" resulting from respiratory changes.

Most parts of the embryo, after the first enlargement of its cells, undergo no further development. The parts of the young plant from which new tissues are to develop must, however, form new cells for a continuation of growth. Therefore the third phase of growth is the formation and enlargement of new cells in the embryonic regions.

### Microchemical

#### *Embryo*

*Sugars.* No reducing sugars can be detected in the embryo of the dry grain, but there is some sucrose, uniformly distributed. The presence of reducing sugar is first detected about 20 hours after the grains are placed in the germinator. This sugar is first found only in the tip of the coleorhiza at the time it is beginning to elongate. The amount of reducing sugar rapidly increases in this tissue, and soon it occurs in all parts of the coleorhiza. When the radicle has broken the sheath and is extended about 3 mm. (this stage is shown in text figure 2), the sugar is present in the now swollen cortical tissue (*c*, text fig. 2) on the face of the embryo, and also in the adjacent part of the radicle. Reducing sugar continues to accumulate in these tissues and in the cells of the elongating radicle, but is never found less than 2 mm. from the tip of the root at any stage.

When the mesocotyl begins to elongate, which is at about the stage when the radicle is 1-2 cm. long, reducing sugar appears in the cells of the cortex of the basal portion of the mesocotyl; there is a gradual increase in amount and in distribution of the sugar, until it is found in all parts of the mesocotyl and in the first node, soon after the plumule has broken the coat. From this time on, reducing sugar is abundant, especially in the cortex and pith of the young seedling. As the seedling continues growth, smaller amounts can be detected in the root and greater quantities are present in the mesocotyl below the first node. Reducing sugar was not found in the cells of the scutellum, except under unusual conditions, although cane sugar was present in about the same amount as in the dry embryo.

*Starch.* In the embryo of the dry grain, starch grains of varying sizes are present in all cells of the scutellum except those of the epithelium, where they are usually absent. Starch grains are also present in the cells of the coleorhiza, coleoptile, and plumule, but apparently not in those of the radicle.

The disappearance of the starch during germination is first noted in the coleorhiza. After about 3 days (when the radicle is 6 cm. long), the starch grains have entirely disappeared from the cells near the broken tip, and

even those at the basal portion of the root sheath show considerable corrosion. The starch grains are not dissolved uniformly nor are they corroded in the manner usually described; after staining with iodine each grain appears as a colorless matrix containing numerous blue dots, giving the appearance of a compound grain. In the swollen tissue on the face of the embryo (*c*, text fig. 2) there is a decrease in the number of starch grains as reducing sugar accumulates in the cells.

Starch begins to appear in the cortex and pith of the radicle when the latter is about 1 cm. long. When the cells of the scutellum begin to enlarge, the number of starch grains within them increases, but there is a great variation in the starch of different individuals grown under apparently the same conditions. Starch occurs in the epithelium only in rare cases, and then as very fine granules.

*Fats.* Fat is probably the most conspicuous inclusion within the cells of the embryo of the dry grain, being especially abundant in the cells of the scutellum and of the epithelium and least abundant in the radicle and mesocotyl. The fat appears as conspicuous globules, and, after treatment with alcoholic Sudan III, it shows a marked tendency quickly to gather in large droplets.

There is a great change in the appearance of the fat after germination starts; it occurs as very small globules dispersed in the protoplasm and shows much less of a tendency to gather in large drops when stained. The fats of the scutellum show no detectable change in amount during four or five days of growth. There appears to be a decrease in the amount of fat in the swollen cells on the face of the embryo (*c*, text fig. 2), where sugar is rapidly accumulating, but this is hard to determine because of the distribution of the fat in a much larger volume of protoplasm.

*Proteins.* There is a very noticeable increase in the intensity of the biuret reaction in the active regions of the embryo, even before the coleorhiza has broken the fruit coat. This would indicate a very early solution or partial hydrolysis of the reserve proteins. Before the cells of the epithelium begin to elongate, this test gives a dull bluish color; but later the color becomes bright purple, then pinkish, and increases in intensity for several days. Under certain conditions, the embryo may remain in the germinator for several days without any apparent elongation of the cells of the epithelium; at these times there is likewise no change in the biuret reaction of the layer. During germination, the biuret test gives a pinkish-purple color in the vascular tissues of the scutellum while other parts of the scutellum show only a dull bluish color. A distinct accumulation of nitrates, as shown by the diphenylamine test, occurs in the mesocotyl just below the first node.

*Cell Walls.* The cell walls of the scutellum are rather thick and clearly show the peculiar sculpturing described by Sachs. The chemical nature of these walls is not certain; they are probably not of one uniform substance throughout, but certainly contain some cellulose. It was not possible to

demonstrate either pectic substances or pentosans. The cell walls of the radicle, mesocotyl, and plumule of the seedling are undoubtedly largely cellulose. The cell walls of the scutellum are not dissolved during germination. Even after the endosperm is entirely digested, the cell walls of the scutellum show no change that can be detected, although the cells are nearly depleted of their reserve materials.

#### *Endosperm*

In the dry grain, the cells of the endosperm are packed with starch grains. In the horny portion, these grains are imbedded in a matrix of proteins. The aleurone layer contains proteins and fat. No reducing sugar was detected in the endosperm. About forty-eight hours after placing on the germinator, reducing sugar first appears in the endosperm. The time of appearance of this sugar does not vary as greatly as is the case in the embryo, nor does there seem to be any connection between the appearance of sugar in the endosperm and the growth of the embryo. The reducing sugar, when first detected, is evenly distributed. As germination progresses, the amount of sugar is greatest near the epithelium of the embryo.

There is evidence of a movement of soluble protein substances from the endosperm toward the embryo, and some indication of proteins passing from the aleurone layer toward the scutellum.

#### *Reaction*

Tests for reaction of the tissue, made with neutral red, indicate that in the dry grain the entire endosperm is distinctly alkaline. The cell walls of the embryo are acid, but the cell content is neutral or slightly alkaline. After germination has started, the cells of the scutellum are distinctly acid, but the endosperm remains alkaline. The sharp line of demarcation in the reaction of the two tissues is very striking.

#### *Oxidizing Enzymes*

Qualitative tests of sections on slides were made for oxidases, peroxidases, and catalase. Neither the dry grain nor seedlings in various stages of development gave a test for oxidases with either benzdine or gum guaiacum. The ungerminated grains gave a moderate reaction on the addition of  $H_2O_2$ , especially the embryos, indicating the presence of a peroxidase. Both the embryo and the endosperm of seedlings gave a marked reaction for peroxidase. The catalase activity of the embryos in both the dry and the germinated grain was very strong, but that of the endosperm was much weaker.

#### *Mineral Elements*

A few tests were made for the presence of mineral elements. The distribution of iron was localized in the seedling. Median cross sections show

it present in the cells of the scutellum adjacent to the epithelial layer, fading out irregularly in the third or fourth layer of cells below. The cells of the epithelium seem to be practically free of iron. Median longitudinal sections of the grain show the same general distribution, except that near the tip of the embryo the iron occurs farther in the parenchyma and also along the vascular tissue of the scutellum. There is no apparent change in its distribution during germination. Calcium was found in appreciable amounts in the cortex of the mesocotyl of seedlings. It was chiefly present along the middle lamellae, but some seemed to be in the protoplasts. Small amounts of potassium and inorganic phosphorus were also found in the mesocotyl of seedlings.

#### Quantitative Chemical Study

The results of the microchemical study were checked by a quantitative determination of some of the material transformations during the early stages of germination. For this work, corn from a single ear was soaked for twelve hours in distilled water and then put to germinate as previously indicated. Samples were taken at three different stages of development; the embryos were separated from the endosperms, and each part was saved separately. In series I there was much unevenness in the early growth of the seedlings, so that at the end of a little less than three days two samples were selected. One sample (*C*) consisted of the grains in which the coleorhiza was just breaking the coats, and the other (*B*) of those farthest advanced and having a radicle 1-2 cm. long. At the end of 5½ days another sample (*D*), consisting of grains having plumules 4-8 cm. long and radicles of the same length, was selected. The germination in series II was much more uniform, as care had been taken to place all grains with the germ side in contact with the filter paper. In this series, sample (*E*) was taken at the end of 42 hours, sample (*F*) at the end of 66 hours, and sample (*H*) at a little less than five days; the stages of development being the same as those represented by the successive samples of series I.

The samples were dried *in vacuo* at 65° C. and stored in glass-stoppered weighing bottles. In series I, the embryos were partially extracted with dry ether and then the residue was ground and re-extracted. In series II, the embryos were ground with an equal weight of pure quartz flour and duplicates were weighed from this material for extraction with ether. It was impossible to grind the embryos directly on account of the large amount of fat. The material after ether extraction was further extracted with cold water, and the residue was dried *in vacuo* and saved for starch determination. It was found necessary to boil the neutralized water extract to prevent enzymatic activity in the solution.

The various determinations were made according to the "Methods of Analysis" of the Association of Official Agricultural Chemists (1912), except that the reducing power of the sugar was determined by the modification of the Bertrand-Munson and Walker methods as given by Mathews

(1919). The amino nitrogen of the water extract was determined by the method of Van Slyke (1912).

The figures given in table 3 are the averages of duplicate determinations. The determinations checked sufficiently closely so that it was not deemed necessary to give them separately. The range of variations of the duplicate determinations is shown by the figures for the percentages of total sugar in samples (E), (F), and (H) respectively: 4.16 and 4.9; 7.0 and 7.2; 16.7 and 16.9.

TABLE 3. *Results of the Quantitative Study of the Transformations of Germinating Maize*

	Series I			Series II		
	(C)	(B)	(D)	(E)	(F)	(H)
Sample.....	67 hrs.	65 hrs.	5½ days	42 hrs.	66 hrs.	5 days
Time under germinating conditions.....	0.2 cm.	1-2 cm.	4-8 cm.	0.2 cm.	1-2 cm.	4-8 cm.
Length of radicle when sample was taken.....						
<i>Embryos</i>						
Dry Weight mg. each	30.5	33.5	70.0	27.1	32.0	55.8
Ether extract.....	Percent	33.9	29.9	10.3	39.4	32.2
	Mg. each	10.4	10.0	7.3	10.7	10.3
	Total { Percent	4.8	6.6	16.6	4.5	7.1
	{ Mg. each	1.5	2.2	11.8	1.2	2.3
Sugar as dextrose.....	{ Percent				3.1	13.7
	{ Mg. each					
	Reducing { Percent			trace		
	{ Mg. each				1.0	7.6
Starch as dextrose.....	Percent			4.5	2.8	3.7
	Mg. each			1.2	.9	2.1
Amino nitrogen of water extract.....	Percent	0.61	0.72	0.93		
	Mg. each	0.18	0.24	0.66		
Total nitrogen of water extract.....	Percent	1.33	2.2	2.53		
	Mg. each	0.48	0.75	1.80		
<i>Endosperms</i>						
Dry Weight mg. each	255.4	255.2	199.5	207.3	199.2	168.4
Ether extract.....	Percent	1.03	1.08	1.35		
	Mg. each	2.65	2.5	2.7		
Sugar as maltose.....	Percent	10.2	10.9	16.3		
	Mg. each	26.1	28.1	33.0		

The very rapid increase in dry weight of the embryo between the third and the fifth days of germination shows how rapidly the reserve material of the endosperm is absorbed by the scutellum. Since this increase is only in small part accounted for by the total sugar, starch, and soluble nitrogen, it is obvious that there must be a very rapid building of permanent cell substance in the embryo from the material absorbed. The loss of fat from the embryo is not great in these first few days, but is at a fairly uniform rate. The rate is greater in series I than in series II.

The increase in sugars is almost entirely in reducing sugar. The very great accumulation of dextrose in the embryo compares well with the results of the microchemical study. A small amount of preliminary work was done to determine the quantitative distribution of reducing sugars in the plumule, in the roots, and in the scutellum. Alcoholic extracts were made of these separate parts of fresh embryos about six days old. The plumules had 7.2 mg. dextrose each, and the roots (including the tissue between mesocotyl and main root) 7.9 mg. each, while the scutellum contained no reducing sugar. It would seem that the sucrose is evenly distributed in the embryo.

The early decrease of starch in the embryo, with a later considerable increase, is based on too few data to draw conclusions, and yet it agrees with the observations in the microchemical study that the starch of the coleorhiza and neighboring tissues shows evidence of solution, and that an increase in the number of starch grains per cell is later noticeable in the scutellum.

The presence and increase of amino nitrogen and additional soluble nitrogen is, of course, what would have been expected.

The small amount of fatty substances and the comparatively constant amount of sugar in the endosperm also agree with the microscopic observations. The sugar of the endosperm has (for convenience) been expressed as maltose, without any attempt to identify the sugar present. There was some increase in reduction after treating as for the inversion of cane sugar, and a further marked increase after stronger hydrolysis. From this, and from the work on cereals by Kjeldahl (1882) and O'Sullivan (1886), it is probable that maltose, sucrose, dextrose, and dextrin are present. It is assumed that in the embryo the reducing sugar is largely dextrose, with the possibility of some fructose; the non-reducing sugar is assumed to be sucrose, as it is very readily hydrolyzed.

### Modified Development

The question of modified development or "predetermination" in germination has recently been made prominent by Kidd and West (1918-19). Their own work shows the marked influence of soaking seeds in water upon the subsequent growth and yield of the resulting plants. From an admirable review of the literature, these authors found abundant evidence, based upon many methods of attack, that the development of a plant may be greatly



modified by the condition and environment of the seed and of the seedling.

In a survey of this problem to learn the most promising line of attack for future studies, some data were obtained as to the modifications in the course of development of seedlings when some of the factors were varied from the conditions taken as standard in the work as detailed above. Although the results are somewhat fragmentary, they are given that they may be available for correlation with other data.

### *Soaking*

Since several investigators, including the writer (unpublished data) have found striking variations in the development of seedlings following a soaking of the seeds in various solutions, it was thought that some knowledge of the variations in the internal changes resulting from the soaking would be helpful in a study of the factors concerned in seedling development.

Corn was soaked, usually for twelve hours, in solutions of various salts of approximately 0.04 molecular concentration. It was then carefully washed and put to germinate in covered glass dishes on filter paper moistened with conductivity water. The dishes were kept in a dark room at 21° to 23° C. Grains, to serve as controls, were put directly in the germinating dishes at the time that the other tests were started to soak. The control grains, which had not been soaked, were always slower and less uniform in starting to germinate, but, after a few days, they showed development equal to that of most of the others. The only constant differences in development were in the lots soaked in  $\text{MnSO}_4$  and  $\text{CuSO}_4$ . In the grains treated with  $\text{MnSO}_4$  solution, the radicle broke the coat distinctly earlier and germination was more vigorous than in the controls. The appearance of sugar was correspondingly earlier and in larger amounts. The radicles of those seeds treated with  $\text{CuSO}_4$  were distinctly retarded in growth, and sugar accumulation was distinctly less than in seedlings from untreated grains. Many individual grains soaked in other solutions showed distinctly retarded development; these retarded seedlings always contained much less sugar than non-retarded seedlings of the same age, and less even than seedlings which had been germinating for a shorter period but were at the same stage of development. Accompanying the retardation of growth in some seedlings, there was evidence of a marked accumulation of starch, instead of sugar, in the embryo.

### *Position*

Great variation was noted in the germination of the individual grains composing a lot placed under supposedly uniform conditions. A correlation was observed between this variation and the position of the germ side of the grain with respect to the moist substratum. Those with the germ placed in contact with the substratum showed the first signs of germination in about twenty-four hours, while those with the germ upward were fully



forty-eight hours in reaching the same degree of development. On the other hand, the absorption of water by duplicate lots of seed in each position was: 32.6 percent and 35.0 percent for those placed face downward, and 28.6 percent and 33.8 percent for those placed with the germ upward. Here, also, the stage of development of the coleorhiza was correlated with the presence of reducing sugar in the embryo, rather than with the length of time in the germinator. In the endosperm, sugar first appeared near the embryo when that side was nearest the moisture of the substratum. Also, elongation of the cells and other evidences of activity of the epithelial layer of the embryo occurred first in the grains with the embryo next the moist paper. When germination was delayed because of placing the grain with the germ side away from the moist substratum, the epithelial layer showed no signs of activity until after the embryo had started growth. In this case, the first development of the embryo seems to have been independent of any activity of the epithelial layer.

In attempting to find the reason for this variation in germination, the tip of each of several corn grains was carefully covered with wax before they were put on the germinator. These grains took up very much less water than untreated grains and were very slow to start germination. The coats of the corn grain, at least when dry, are obviously not readily permeable to water, as has been assumed, but the first water seems to enter the fruit through the stalk and vascular tissue which had originally connected the developing grain with the parent plant. This is similar to the condition in the case of the seeds of several legumes, as noted in some unpublished work by the writer.

#### *Oxygen*

The first stages of germination of corn in sealed flasks of varied volume did not differ especially from that in petri dishes, but the extent of growth finally attained corresponded with the volume of air available. The failure of growth was not associated with a lack of sugar-formation; in fact, there was an unusual accumulation of sugar.

Mazé (1900) noted that the production of sugar is not stopped in maize by limiting the oxygen supply during germination. He attributed the checking of germination to the inactivity of oxidizing enzymes. On the other hand, Boysen-Jensen (1912) finds that oxygen is necessary for the production of cane sugar in the barley seedling. The exact relation of the seedling to oxygen, as well as the course of respiration during germination, needs careful analysis.

#### *Excised Embryos*

The endosperms were removed from dry grains, and in some cases a part or all of the scutellum was also removed. The embryos were then put under conditions for germination. The early stages of germination progressed as in unmutilated grains; the coleorhiza, radicle, and plumule began to

elongate after about the usual lapse of time. The amount of growth of the roots beyond a length of approximately one centimeter is checked to a marked extent when the food reserves of the endosperm are not available. The elongation of the mesocotyl and the development of the plumule are affected to a much less extent by the absence of the endosperm. The early appearance of reducing sugars in the growing parts was the same as in the seedlings from the entire grain, except that reducing sugar was often found in the cells of the scutellum. The sugar greatly decreased in the radicle after this part was checked in its growth, but persisted in the elongating mesocotyl and at the first node. The fat of the scutellar cells showed a great decrease, in marked contrast to the condition in other seedlings of the same age, or of the same stage of development. Sometimes there was a noticeable decrease in the amount of starch in the scutellum, but this was not at all constant.

Grains from which the complete growing axis, including the plumule and radicle, had been removed imbibed water as usual and showed the presence of some sugar in the endosperm after about twenty-four hours, but even after a week or more there was no indication of activity of the epithelial layer and no accumulation of sugar in that part of the endosperm near the scutellum.

The diminution in size and number of the vascular elements, as found by Urbain (1920) when the developing seedling is deprived of the reserves of the endosperm, is probably sufficiently explained by the limitation of the available building material, although the possible effect of the more rapid utilization of the fat and the limited supply of proteins should also be considered.

No definite experiments were planned to determine the effect of temperature on the course of development, but some incidental observations indicate that the entire course of internal changes may be greatly modified with variations in the temperature of germination.

Gassner (1910, 1918) has observed that the development of winter cereals is dependent upon the temperature during their early growth. If the temperature for germination is lowered to 1° C., winter cereals planted in the summer proceed to develop to maturity at once; when germinated at a high temperature, the plants remain dwarf and leafy during the entire summer. Facts concerning the variation in metabolism of seedlings germinated at different temperatures should throw interesting light on this problem and on the factors involved in the correlations of plant growth.

#### DISCUSSION

The correlation of the appearance of reducing sugar in the embryo with the first growth of the tissue in which such appearance occurs, and the sharp localization of these changes, illustrate the dependence of growth on internal changes. The conditions which initiate these changes, and which

keep them localized first to one group of cells and then to another as germination progresses, deserve further investigation. It would seem that, in many cases at least, the first growth of the embryo begins in advance of any changes in the endosperm. I believe, with Sachs (1862*b*), that the embryo utilizes its own stored food for its initial growth changes. The dextrose, which appears in the embryo before changes in the endosperm are evident, may have its origin from the sucrose, from the stored fat, or from the starch present in the embryo. At any rate, the starch in the coleorhiza shows evidence of digestion before the movement of sugar from the endosperm to the growing axis begins, although such movement of sugar takes place at a very early stage.

To explain the impossibility of detecting reducing sugars in the scutellum, although the digested carbohydrates of the endosperm must pass through that tissue to reach the radicle and plumule, is as puzzling as when this fact was first observed by Sachs. Rywosch (1909) believes that the translocation of reserves of both leguminous and grass seedlings depends on the concentration gradient maintained between the cells where digestion of reserves takes place and the tissue where the food is utilized. However, such an explanation does not account for the great accumulation of sugar in maize in the cells where it is to be used. It is possible that the sugar is translocated so rapidly that it can not be detected, or that it occurs in the cells of the scutellum in a colloidal form or in combination with some other substance, possibly with the proteins, so that it does not have reducing properties. Boysen-Jensen (1915) states that both monosaccharides and disaccharides may appear as the translocation form of carbohydrates in seedlings of the pea; thus it is also possible that the sucrose, constantly present in the scutellum, may represent the translocation form of carbohydrates in maize.

It is impossible to state whether the accumulation of sugar in the cells of a tissue is directly related to the growth of those cells in which such accumulation occurs, or whether such an association is incidental. Undoubtedly, the sugar causes an increase in the osmotic pressure of the cells in which it occurs, and the dextrose present also certainly furnishes a material readily available for use in respiration; but neither of these suggestions would account for its accumulation and its persistence in such large amounts. Eckerson (1913), in a study of the changes accompanying the after-ripening of seeds of *Crataegus*, noted the appearance of sugar in the hypocotyl, as one of the conditions accompanying the later stages of preparation for germination. Choate (1921) found this same early appearance of reducing sugar in the coleorhiza of germinating wheat. Most of the other work on the chemical changes occurring during germination has not dealt with these early stages. The additional observation that poorly growing seedlings usually have a very much smaller amount of sugar in the growing axes suggests that further work may disclose some important relation between the factors involved in sugar accumulation and growth.

Sucrose is present in the embryo in comparatively small and rather constant amounts during germination. The data of Brown and Morris (1890) and of O'Sullivan (1886) would indicate a larger proportion of sucrose in the developing embryos of barley and of wheat than in that of maize.

Maize differs markedly from wheat in having a large amount of fatty substance in the embryo. Its occurrence to the extent of almost forty percent of the dry weight of the whole embryo would suggest that it is a major source of reserve food for the seedling, but as a matter of fact its utilization in the early stages of growth is limited. Miller (1910) has shown that the fats of the sunflower seed are the source of the sugar accumulated in the growing hypocotyl, but even in this plant the depletion of the fatty reserve is very gradual. In maize, the starch of the endosperm would seem to be a much more readily available source of food for the developing plantlet than the fat of the embryo itself. It was not determined whether the fats are hydrolyzed before translocation, as there is no satisfactory microchemical test now available for the differentiation of fatty acids from the neutral fat. Miller (1912) has shown the marked increase in the acid number from the neutral of the ether extract during the progress of germination of *Helianthus*, but such increase was not appreciable until after about seven days of growth. The presence of nearly  $1\frac{1}{2}$  percent of cholesterol and a like amount of lecithin in the oil of the corn embryo, as shown by Hopkins (1898) and by Winterstein and Wünsche (1915), may have a considerable influence on the water relations and active metabolism of the tissues of the embryo. Since in the excised embryo, deprived of the starchy reserves of the endosperm, a considerable early growth occurs at the expense of the stored fats, it is obvious that a mechanism is present for its ready digestion and utilization.

The changes of nitrogen compounds during germination are difficult to follow, and little information has been obtained that adds to previous conceptions of the internal processes of the plant. The very early increase in the intensity of the biuret reaction in the active regions of the embryo would indicate the partial cleavage, or at least the making soluble, of the proteins already present in these parts. On the other hand, there seems no doubt but that the large amount of protein stored in the horny part of the endosperm is drawn upon by the developing plantlet. Osborne (1909) considered the "reserve proteins" of the seed to be largely those occurring in the endosperm. The proteins in the embryo were held to be more nearly comparable to the "tissue proteins" of animals. The work of Chittenden and Osborne (1891-92) and Osborne (1897, 1909) on the proteins of the entire maize kernel, and of Winterstein and Wünsche (1915) on the protein of commercial "germs," showed that the embryo contains a small amount of globulins and a larger amount of alkali-soluble protein, while the endosperm contains zein and globulins. Since Andronescu (1919) has found it possible to grow seedling corn plants of sufficient vigor to attain maturity,

although the embryos had been removed from the endosperm before germination, there must be a considerable supply of proteins in the embryo which can be utilized for the early growth of the seedling. It is not known to what extent the seedling is dependent on the reserve proteins of the seed, or how soon the young plant is able to synthesize amino-acids and proteins from simpler materials.

Urbain (1920), who has investigated in many seeds, including maize, the influence of the presence of the endosperm on the development of the embryo, found that the effect of the endosperm is especially marked during the first few days of germination. The actual amount of food absorbed from the endosperm by the embryo, however, during this time, was small; because of this fact, he suggested that the "endosperm is as effective by its presence as by the actual food furnished." It is possible that this early dependence of the embryo upon the endosperm for vigorous development is due to the relative proportion of carbohydrates and proteins available to the growing regions, or to the concentration of solutes in the embryonic tissues of the seedling.

Haberlandt (1890) and others have suggested that the embryo is dependent on the endosperm for nitrogenous material for the building of diastase. The fact that Dubard and Urbain (1913), Urbain (1920), and Andronescu (1919) have noted differences in the course of development of maize and other embryos deprived of their endosperm, might indicate that the type of protein available during the first few days of germination has an important influence upon the future development of the seedling.

Winterstein and Wünsche (1915) were unable to detect nucleic acids in commercial maize germs, although the embryos of wheat yield large amounts. However, it seems hardly probable that they are not present in corn; it is likely rather that the method of extraction was not suitable for these substances, or that they may have been destroyed in commercial degermination. In fact, Zaleski (1911) reports the presence of nucleo-proteins in maize and their general occurrence in seeds.

The exact method of the depletion of the endosperm during germination and the subsequent growth of the seedling does not seem to be settled in spite of all the work that has been done. My own observations agree with those of many others that it is possible for sugars to appear in the endosperm without any evidence of activity of the epithelial cells. I also agree with Mann and Harlan (1915) that all the evidence indicates that in normal germination the absorption of the endosperm always starts next the scutellum and progresses toward the more distant parts of the grain. Sugars were never found in large quantity near the scutellum, nor was there actual depletion of the endosperm tissue without clear microscopic evidence of activity of the epithelial layer. The evidence is strong that the epithelial layer is the main source of enzymes for the digestion of the materials composing the endosperm, as well as being an absorptive organ, although the

facts so far available do not furnish satisfactory proof on this point. A question of more interest is the apparent dependence of the activity of the epithelium upon the previous growth of the axis, as shown by several different observations. It is possible also that the development of enzymes may in part depend upon the absorption of proteins derived from the endosperm, as suggested by Brown and Morris (1890) and Haberlandt (1890). A more intensive study of the interrelations of the various activities of germination will advance the physiology of germination a great deal further than all the work that has been done as a basis for arguing the question as to whether the endosperm reserves are dead or alive.

The main results of the present study emphasize the close interrelation that exists between the various internal processes concerned in germination, and also the sharp localization of the chemical transformations which may be associated with such processes. It is clear that it is impossible to make an interpretation or simple statement concerning the factors which control germination before sufficient facts are available detailing the exact relations of the different processes concerned. It is necessary, therefore, greatly to extend the amount of data beyond those now available for analytical interpretation, through the institution of experiments the results of which would be capable of examination from this viewpoint.

I wish to take this opportunity to express my great indebtedness to Dr. E. J. Kraus of the Department of Botany of the University of Wisconsin for his kindly interest and helpful counsel throughout the progress of the work, and to Dr. W. E. Tottingham of the Department of Agricultural Chemistry for help, especially with the quantitative analyses. Dr. S. H. Eckerson of the University of Chicago also has kindly helped in checking the microchemical results.

#### SUMMARY

1. The endosperm of corn has stored reserves of carbohydrates and proteins. The embryo contains about forty percent of fat, some starch, and about four percent of sucrose, in addition to proteins.
2. Following imbibition, the next evidence of germination is the swelling of the coleorhiza until it breaks the fruit coat. The subsequent elongation of the radicle breaks through the coleorhiza. Later the elongation of the mesocotyl pushes the developing plumule through the coat. At about this same time, the secondary roots begin to develop.
3. The elongation of the coleorhiza is accompanied by the appearance of reducing sugar in the enlarging cells. As the enlargement of cells composing other tissues begins, reducing sugar is found in the enlarging cells until it is found in all growing parts except the embryonic tissue.
4. Non-reducing sugar is present in all parts of the embryo during all stages.
5. There is a correlation in most cases between the enlargement of cells and their sugar content.



6. The decrease in the amount of fat contained in the embryo is slow and gradual.
7. Soluble proteins move from the endosperm to the active tissues of the embryo.
8. Reducing sugar appears in the endosperm at about the same time as, or somewhat later than, in the embryo.
9. Depletion of the reserves of the endosperm begins next the scutellum and progresses into the endosperm. At the end of seven days, only a small part of the endosperm reserves have been used.
10. There is evidence of correlation of the activity of the epithelial layer with growth changes in other parts of the seed.
11. The early germination of embryos deprived of their endosperms shows the same changes as in the intact grain, but the later development is modified by the nature of the food supply.
12. The evidence from both microchemical and quantitative methods is in complete agreement.
13. The above-outlined course of development may be modified by variations in the external environment.

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## THE PHYSIOLOGY OF POLLEN III. GROWTH *IN VITRO* AND *IN VIVO*<sup>1</sup>

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### THE LENGTH OF POLLEN TUBES GROWN ON ARTIFICIAL MEDIA AS COMPARED WITH THE DISTANCE FROM RECEPTIVE SURFACE TO OVARY IN THE PLANT

It was stated in an earlier paper in this series that the amount of growth of pollen tubes grown *in vitro* was, except in a few forms, almost negligible in amount when compared with the distance the tube must travel in the plant in order to effect fertilization. This fact has been emphasized by numerous workers, some of whom have called in question the value of artificial cultural studies on this account. It is a fact that even successful germination has been found difficult with pollen of many forms, and the subsequent growth in others that germinate freely is very feeble and irregular. Much of the variability in the behavior of pollen from forms that will from time to time give very satisfactory results remains to be accounted for. But the problems are not unapproachable; the insight already gained into some phases is no cause for apprehension.

An encouraging feature of the studies on methods of culturing pollen artificially has been the discovery of a few species that will produce tubes *in vitro* comparable in length to those growing on the style of the plant. Bobilioff-Preisser (1917),<sup>2</sup> working with *Vinca minor*, a form in which the styles are 0.5 to 0.8 cm. in length, secured pollen tubes about 1.0 cm. long on 1.5-percent agar media containing from 5 to 10 percent cane sugar. The writer has worked rather extensively with *Vinca minor* pollen and has secured some tubes as long as the style on sugar-agar cultures containing a little sterile yeast. Knight (1917) reports that about 5 percent of the pollen tubes of apple growing in a 3-percent fructose solution containing

<sup>1</sup> Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

<sup>2</sup> References are to literature cited at the end of Part IV, to appear in a future issue of this JOURNAL.

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traces of asparagin attained a length of 10 mm., this being more than the required length to reach from stigma to egg. The writer has attempted to repeat this test but without success. Pollen from a number of species of *Pyrus* growing in the Arnold Arboretum was used, and a variety of media were tried. Usually good germination was secured, but in no case did the tubes reach a length of over 2 mm. We have no satisfactory explanation to offer for this difference.

The present writer has found that pollen from four species of the family Liliaceae will produce tubes on artificial media long enough to effect fertilization if they were growing in the style of the plant. On a 1.5-percent agar medium containing 10 percent cane sugar and a little yeast, excellent growth of the pollen of the grape hyacinth, *Muscari botryoides*, was secured. In the grape hyacinth the styles are about 1.25 mm. in length, and the distance from the stigma to the base of the ovary is about 2.5 mm. but occasionally reaches 3.0 mm. The majority of the tubes on the artificial medium were as long as the style, and a few approached the length of the vertical axis of the gynoecium. The lengths of some of the longer tubes in millimeters were as follows: 1.65, 2.30, 2.04, 1.79, 1.67, 2.02, 1.59, 1.79, 2.20, 1.77.

In *Puschkinia*, the styles average about 1.5 mm. in length. On a culture medium consisting of 1.5 percent agar, 7 percent cane sugar, and a little sterile yeast, good growth was obtained. The following measurements in millimeters were taken on the longer tubes: 1.41, 1.51, 1.55, 1.57, 1.69, 1.63, 1.55, 1.43.

On the same culture medium, the longer pollen tubes of *Chionodoxa*, a form in which the style is about 2 mm. long, measured as follows: 2.04, 1.77, 2.18, 1.91, 1.96, 2.00, 2.01 mm.

Excellent growth of pollen tubes of *Scilla* was obtained on a culture medium consisting of 2 percent agar, 7 percent cane sugar, and a little sterile yeast. Careful measurement showed that the styles of this species range in length from 4.5 to 6.0 mm. The pollen was scattered at random on the culture, and the tubes were grown at room temperature. Fourteen tubes measured as follows: 4.96, 5.14, 5.10, 5.40, 5.34, 4.98, 5.00, 4.92, 5.22, 5.16, 4.75, 6.12, 4.68, 5.40 mm. On this culture there were many shorter tubes, but a very considerable number equal in length to, or even longer than, those whose measurements are given above.

#### NUCLEAR BEHAVIOR

On the plant, presumably the sole function of the pollen tube is to provide a means of approach of the passive or almost passive sperm nuclei to the embryo sac. The pollen grain germinating upon the stigma gives rise to a pollen tube into which the so-called tube and generative nuclei (or sperm nuclei) pass and make their way to the ovary as the tube extends through the stylar tissue. Stated in these terms, we may say that the

results of our studies on artificial media are successful in so far as they promote this nuclear migration. It has already been demonstrated that tubes of sufficient length to reach from stigma to ovule can be secured *in vitro*. It remains to be seen if these have brought their nuclei in position to function were fertilization possible.

Few observations have been made on the behavior of the nuclei in pollen tubes growing on artificial media. The density of the protoplasmic contents of the tubes, with their frequently coarse granular inclusions, makes it usually difficult or impossible in whole mounts to detect the nuclei with certainty. Van Tieghem (1869) was able to get embryos showing the first stages of development on artificial cultures. Ovules in the path of pollen tubes received them through the micropyle and some development ensued. Strasburger (1878), in similar experiments with *Torenia*, observed fertilization *in vitro*. Elfving (1879) was successful in securing short tubes of several species on sugar media and found that these usually contained two nuclei in their tips. In the pollen tubes of *Plantago media*, Elfving observed and figured three nuclei, one nearest the tip long and narrow, the two others closely behind it, small and oval in shape. The lengths of the tubes in these cases are not given.

In Scilla, a form in which tubes as long as the style of the plant can be secured on agar cultures containing 7 percent cane sugar and a little sterile yeast, the writer found excellent material in which to follow the nuclear behavior in pollen tubes growing *in vitro*. The cytoplasm in the grain and in the tube is very clear. By using Belling's (1921) aceto-carmine, which stain was kindly suggested by Dr. Karl Sax, the large nuclei staining a deep red are sharply differentiated from the more lightly colored cytoplasm.

Observations were made on fresh pollen grains and on tubes at intervals during their growth by removing these from the cultures with a needle and mounting them in the stain. The drawings were made in part with the aid of the camera lucida; the remainder were sketched free-hand. In fresh pollen, as is shown in figure 1, Plate XIX, usually only a single deeply staining nucleus can be detected with certainty. Figure 2 represents the less frequent condition, in which the second and smaller nucleus, although staining faintly, can also be seen. At the time the pollen is shed it is very probable that two nuclei are already differentiated in the grain, although one stains more readily than the other. At the onset of germination the second nucleus is aroused to activity. Usually within 15 minutes after the pollen grain has been placed on the culture the tube is emitted. At this time two nuclei may be clearly observed in the grain (figures 3 and 4). Migration of the nuclei, as shown in figure 5, commonly begins at one hour, or when the tubes have attained a length of about 100  $\mu$ . Except in rare cases, the smaller nucleus, presumably the tube nucleus, leaves the grain first and is followed closely by the larger generative nucleus.

In figure 12 are shown pollen tubes pressed out of the stigma of Scilla

one hour after pollination. Here, as in the artificially cultured tubes of the same age, both nuclei take the stain and migration has begun. Attempts to follow the pollen tubes in their further growth in the style were unsuccessful, but we may reasonably suppose that in this species the nuclei behave as in the closely related forms which have been studied.

On artificial cultures  $1\frac{1}{2}$  hours old, many of the tubes have reached a length of  $300\ \mu$ . At this stage the tube nucleus is found a short distance from the tip, and the generative nucleus, frequently vermiform in appearance, lies free in the body of the tube as represented in figure 6. In many cases at 5 hours, elongation has ceased and the two nuclei, one closely behind the other (figure 7) are found in the somewhat enlarged tip. Figure 8 represents the tip of a tube about 4 mm. in length taken from a culture after 6 hours. Here the rear nucleus has divided, giving rise to two vermiform sperm nuclei. These do not appear to be accompanied by any specially differentiated cytoplasm. A similar condition is seen in text figure 4, a microphotograph of the nuclei in a *Scilla* tube 4.24 mm. in length. Here the tube nucleus was 0.37 mm. from the tip of the tube. Each of the sperm nuclei measured about  $30\ \mu$  in length. No details concerning the division of the generative nucleus to form the two sperm nuclei were obtained. At 7 hours, as shown in figure 9, Plate XIX, in those tubes in which the sperm nuclei have been formed these have become more compactly organized and the tube nucleus is passing into a "resting condition."

In some tubes on 7-hour cultures, various aberrant distributions of the nuclei have been found. A tube 1.37 mm. long is represented in figure 10, one nucleus of which has failed to leave the grain. In another tube (figure 11) 1.43 mm. in length, two nuclei were found near the tip and a third lying near the grain end. In the cases in which the tubes reached an ultimate length equal to only a few diameters of the pollen grain itself, the nuclei usually still remain in the grain. In some instances either the smaller or the larger nucleus is found in the tube, but very seldom both. The relation of nuclear position to pollen-tube length is difficult to analyze. Since, however, some short tubes are found in which the nuclei have taken up their usual position, the writer is inclined to the opinion that the failure of the nuclei to migrate is not *per se* responsible for the limited amount of growth these shorter tubes exhibit. On older cultures pollen grains are occasionally found that have not germinated, in which the nuclei are to all appearances quite normal. Here, as it appears in the shorter tubes, the position of the nuclei is a consequence and not a cause of the failure of the tube to elongate. In a rapidly growing tube, however, where the activity is centered in the tip, we might expect, if the tube nucleus failed to advance to its usual position, that its influence on this growing region would gradually diminish and that growth consequently would be retarded.

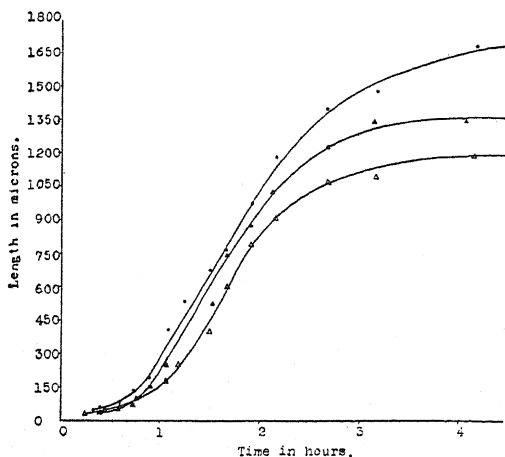
The observations as a whole indicate very close agreement between the behavior of the nuclei of pollen tubes growing *in vitro* and those growing *in*



*vivo*. In the majority of the tubes growing on the artificial medium, the nuclei migrate into the tubes in the early stages of development and thereafter maintain a relation to the growing tip similar to that found in the styles of such other Liliaceae as have been examined by various workers. On the artificial cultures, moreover, division of the generative nucleus to form the two male gamete nuclei in the longer tubes has been frequently observed. The possession of these facts on nuclear behavior lends further support to the view that culture *in vitro* is a rational method of attack upon the problems of pollen physiology.

#### GROWTH CURVES

If some favorable form be chosen, and the pollen grains be plated in such a way that each may be quickly and certainly identified, the length of

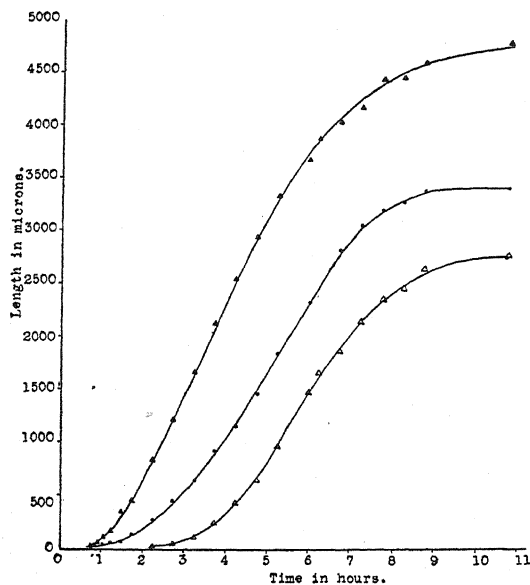


TEXT FIG. 1. Growth curves of pollen tubes of *Scilla* grown on an artificial medium consisting of 2 percent agar, 10 percent cane sugar, and a little sterile yeast, grown at an oven temperature of 27°–28° C., and measured periodically at a room temperature slightly lower.

each of a series of tubes formed can be determined at intervals throughout the growth period. When the values for length are plotted as ordinates over those for time as abscissae, an S-shaped curve of growth is obtained. Pollen of *Scilla* and *Vinca* was found quite suitable for this work. Each grows readily on artificial media and pursues a fairly direct course during elongation, facilitating accurate measurement. The cultures were grown in the oven at 27°–28° C., being removed to the stage of the microscope and measured at room temperature. This was subject to some fluctuation, but was generally somewhat lower than the oven temperature. The interval required for measurement being comparatively brief, it is believed that the periodic removal of the cultures from the oven did not alter the character of the growth to an appreciable extent.



In *Scilla*, the modal length of time required for pollen-germination is about 20 minutes. In a series of 25 pollen grains taken at random, for example, 6, or 24 percent, germinated within 15 minutes after planting, 10 others, or 40 percent of the total, germinated within 20 minutes, 5, or 20 percent, required 25 minutes, and 2, or 8 percent, germinated in 45 minutes: the remaining 2 grains failed to germinate. A potent factor in determining

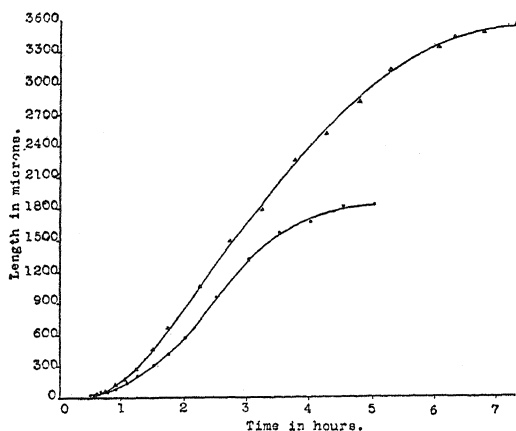


TEXT FIG. 2. Growth curves of pollen tubes of *Vinca minor* grown on an artificial medium consisting of 1.5 percent agar, 10 percent cane sugar, and a little sterile yeast. The tubes were grown at an oven temperature of 27°-28° C. and measured periodically at room temperature, generally slightly lower.

the variability in ultimate length of pollen tubes growing *in vitro* is the bursting of the tubes during elongation. Tubes in any stage of growth may suddenly explode at the tip, and of course cease to grow further. Up to the time of bursting, these tubes can not be distinguished by their behavior from those which survive them. In text figure 1, the growth curves are given of three tubes of *Scilla*, constituting a fair sample of those whose elongation was not terminated by bursting. They were grown on a culture medium consisting of 2 percent agar, 10 percent cane sugar, and a little sterile yeast.

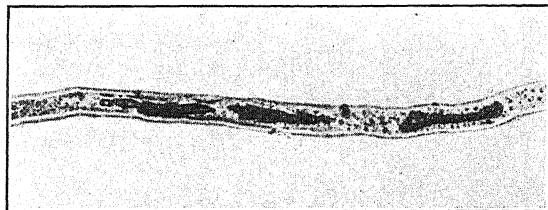
While the ultimate length reached by each of the three tubes represented in text figure 1 differs somewhat, and while there is some variability between them in rate of growth, the type of growth curve given by each is essentially the same. It resembles that of a monomolecular autocatalytic reaction where the amount of substrate is limited. The rate is slow at first, then

becomes more rapid, and slows again. There is some tendency toward asymmetry in these curves, the diminution in rate in the later stages being more gradual than the acceleration in the early stages. And, contrary to expectation if the growth follows strictly the course of a monolecular autocatalytic reaction, the maximum rate of elongation does not occur when growth is just half completed. It comes somewhat earlier than this.



TEXT FIG. 3. Growth curves of pollen tubes of *Vinca minor* cultured *in vitro*, the lower curve representing growth in a yeast-free medium and the upper one growth on a medium containing sterile yeast.

Growth curves were also obtained for the pollen tubes of *Vinca minor* growing at 27°–28° C. on a 1½-percent agar medium, with 10 percent sugar and a little sterile yeast added. In the particular test which yielded the data used in the construction of the curves given in text figure 2, 14 pollen



TEXT FIG. 4. Microphotograph of a section near the tip of a *Scilla* pollen tube grown on a 2-percent agar medium containing 10 percent sugar and a little sterile yeast and mounted directly in aceto-carmin. This tube was 4.24 mm. in length; the tube nucleus (somewhat dumbbell-shaped) was 0.37 mm. from the tip.  $\times 300$ .

grains selected at random were planted in series. Of these 14 grains, all germinated but one, but 7 of the tubes burst during elongation. The remaining 6 gave rise to tubes which maintained their integrity until their growth cycle had been completed. The three curves given are representa-

tive of these latter. As in the case of *Scilla* pollen tubes, the growth curve of *Vinca minor* tubes grown on artificial media simulates that of a monomolecular autocatalytic reaction where the amount of substrate is limited. The significance of the facts presented here in regard to the type of growth prevailing among pollen tubes growing *in vitro* will be considered in conjunction with our observations on the digestion of the reserve materials of the pollen grain during growth, as set forth in the next section.

Further observations reveal the fact that the presence or absence of sterile yeast in the media does not affect the type of growth given by pollen tubes. In text figure 3, growth curves of representative *Vinca* tubes from yeast-containing and yeast-free media are compared. In the presence of yeast a greater amount of growth is given, but from the character of the curves it is evident that the type of growth is not changed.

#### WATER IN ITS PHYSICAL AND CHEMICAL RELATIONS TO GROWTH

There is considerable evidence to show that in some forms the rate at which water is delivered to pollen is the most important factor in germination. Jost (1905) found that pollen of *Dactylis*, *Arrhenatherum*, *Secale*, and *Glyceria*, which could not be induced to form tubes when placed directly on culture media, germinated in a damp room when placed near the media. An examination of the stigmas of these plants confirmed Jost in his idea that the water relation was the most important cultural condition. When viewed under the microscope, no drop of liquid was in evidence on the stigma but only a thin layer at the point of contact with the cover glass. The fact that pollen of *Secale* and *Dactylis* germinated on the stigmas of various other species of plants and on the under sides of some leaves, Jost regarded as evidence that germination is dependent upon definite physical conditions. In a later paper, Jost (1907) reports that in *Corydalis* the only requirement for pollen-germination is a careful control of the water supply. Martin (1913), working with the pollen of *Trifolium pratense*, found that while sugar solutions of various concentrations gave unsatisfactory results, germination could be secured on hog's bladder previously soaked in sugar solutions or in distilled water. Differences in thickness and texture in different parts of the membranes used caused a lack of uniformity in germination. These facts led Martin to the conclusion that the amount of water supplied is the controlling factor in germination. Martin found that the exposed portions of the papillae on the stigmas of *Trifolium* are covered with a rather heavy cutinized wall. An oily emulsion, such as Strasburger (1884) found in the cuticular cells of the stigmas of Gramineae, was found in these structures on the clover plant. These findings led Martin to the conclusion that "the behavior of the stigma indicates that its function is to regulate the water supply and the nature of the pollen necessitates no other function." Anthony and Harlan (1920), prompted by the results of careful observations in the field at pollination time, were able to germinate

the very sensitive pollen of barley by placing it on a slide inside a Van Tieghem cell where moisture was supplied either from a drop of free water or from a piece of mesophyll from the leaf of a garden pea. After the cover glass was put in place the slide was placed out-of-doors on a window ledge. While the humidity of the atmosphere within the cell was coming into equilibrium it was expected that favorable moisture conditions would be met, some moisture would condense on the pollen, and germination would ensue. Under these conditions pollen tubes were obtained in five minutes. Martin (1915), in studying the problem of seed-production in alfalfa, found the pollen of this form very sensitive to moisture. Martin concluded from the results of his experiments that the requirement for germination of the pollen depends upon a certain ratio between the moisture delivered by the stigma and the moisture of the air surrounding the stigma.

Under conditions where the amount of water delivered to the pollen of the above-named forms is not held within narrow limits, bursting usually results. This, as we have discussed in another section, is considered as predominantly an osmotic phenomenon although not entirely independent, perhaps, of the imbibitional processes of the pollen grain. It is quite probable that in the above-mentioned cases the excess water exerted injury by its purely physical effect.

In the culture of pollen *in vitro*, the water in the medium must also be considered in another fundamentally important connection, namely, as a component in the hydrolytic and synthetic reactions that attend growth. In following the digestion of the reserve materials by microchemical means in the pollen tubes of *Vinca minor* during growth, the interesting fact was revealed that when the longer tubes have reached their maximum length the reserves are either greatly diminished or have disappeared entirely. In Plate XX, figures 17-23, successive stages are represented in the digestion of the fatty reserve material characteristic of the pollen of *Vinca*. The tubes were grown on 2-percent agar media containing 10 percent cane sugar and a little sterile yeast. At intervals throughout the period of elongation, tubes were removed from the surface of the agar culture and mounted directly in Sudan III. A sharp differentiation of the fatty particles was thus obtained. A large number of tubes were examined, and, as would be expected, some variability was in evidence among those of a given age both as to length and as to the amount of fatty substance present. In those tubes which fail to grow for more than one or two hours, comparatively little diminution in content of reserve material takes place. Our attention was largely confined to the longer tubes, and the figures given can be considered as fairly representative of the general condition prevailing among these at the various stages.

Germination usually takes place about 45 minutes after the pollen is placed on the medium. Following germination, the coarsely granular contents of the pollen grain pass into the tube as it elongates. Figure 17

represents a tube about 15 minutes old. At this stage the short tube is densely crowded with particles of fatty material of various sizes. While there may be some further accumulation of fat at this time, we are inclined to think that the amounts observed in the tubes are no greater than could be accounted for by the contents of the pollen grain. Thirty minutes later, as is shown in figure 18, many of the smaller fat globules have coalesced, and these larger particles are tending to accumulate toward the tip. From this stage on, the pollen tubes become somewhat more attenuated in appearance, the cytoplasm of the grain and the older portion of the tube gradually becoming clearer through the localization of the fat globules in the region of the tip. In figures 19-23 only the tips are shown, the remaining portions of the tube, as shown in figure 13, being almost or entirely free of granular material. It is readily evident from an examination of figures 17-23, covering a period of about five hours, that there is a diminution progressing with age in the amount of fat in the tubes. Figures 21 and 22, representing tubes from cultures six hours old, show a condition approaching the complete digestion of the stored material contributed by the pollen grain. These reserves are being broken down and utilized in the growth processes. In figure 23 a tube is shown of the same age, in which the fat has entirely disappeared. It is an interesting and significant fact that in such tubes little or no further elongation takes place. Many tubes on these *Vinca* cultures cease to elongate before their reserves are fully utilized. Factors other than deficient food supply intervene to check growth in such cases. The most frequent cause of the premature cessation of growth is bursting. When a tube becomes broken at the tip, its contents are lost in the medium and its elongation is abruptly halted. The point to be emphasized, however, is that the amount of growth of those which continue to elongate appears to be terminated eventually by the exhaustion of the supply of fatty substance in the tube. Under the conditions of culture *in vitro*, potential length of the tube and the amount of food material initially present in the pollen grain seem to be causally related.

In order to evaluate these findings in regard to the digestion of the reserve food material, it is necessary to bring in review certain facts concerning the kind of pollen-tube growth prevailing on artificial media and in the style of the plant as revealed by the type of growth curve obtained in each case. The studies of East and Park (1918) on the rate of pollen-tube growth in the style of *Nicotiana* revealed the fact that after a compatible cross the curve obtained by plotting lengths of tube as ordinates and time as abscissae simulated that of an autocatalytic reaction. In the writer's opinion it would be justifiable to add "where the substrate was present in excess," since the rate of growth in the later stages shows no sign of diminution; the curves so far as plotted do not "turn over." In some successful grafting experiments, Jost (1907) demonstrated that pollen tubes would grow through two styles placed end to end; that is, pollen tubes can grow

much longer than is necessary to effect fertilization. There is no reason to suppose that the position of the pollen tube in the style has any influence, *per se*, on the rate of growth. In so far as the physical impediments interposed by grafting would permit, it is very probable that in Jost's experiments the tubes traversed the styles in a single cycle of growth. If the rate were diminished as the tubes neared the end of the first style through a lack of food material, it would be unlikely that they could have grown through and even projected beyond the second one. The evidence afforded by Jost's experiments and those of East and Park support the view that in the style of the plant there is no diminution in rate of growth in the later stages such as would be expected were there a deficiency of substrate.

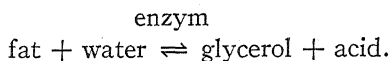
As shown in a previous section, the growth curves of pollen tubes grown on artificial media resemble that of an autocatalytic reaction, which, according to the commonly accepted interpretation, is slower in the initial stages because of a deficiency of catalyst and in the final stages because of a deficiency of substrate. One further fact is to be noted here, namely, that as shown in *Vinca minor* the reserve material of the tube is practically exhausted when the tube has reached its maximum length. Loeb (1906) contended that the synthesis of nuclear material, a conspicuous feature of growth by cell division, is an autocatalytic process and that the nucleus itself or one of its constituents acts as the catalyser. Robertson (1908 *a, b*) expanded this idea and sought to show that data on the growth of man, of *Cucurbita pepo*, and of fresh and dried oats are graduated in a satisfactory manner by the curve for an autocatalytic reaction. It is concluded that the agreement found is good, and Robertson sets forth the view that, in all probability, cell growth, or the synthesis of cytoplasm, is an autocatalytic reaction.

It has not seemed pertinent with the somewhat limited amount of data available at this time to determine if satisfactory graduations are obtained when the theoretical curve for an autocatalytic reaction is fitted to the observations on pollen-tube growth *in vitro*. The evidence presented, however, is suggestive that this type of curve may adequately describe the quantitative relations of the phenomenon. Pearl (1909) has pointed out the fallacy of supposing, in the absence of confirmatory evidence of another sort, that phenomena involving quantitative changes described by the same type of curve have a common cause; qualitative proof must be furnished to establish finally the qualitative identity of phenomena. The growth curves presented above, representing graphically the quantitative relations obtaining in pollen-tube growth on artificial media, are supplemented by observational evidence on the digestion of the reserve food material during elongation of the vegetative cell. There is little doubt that growth is causally related to the digestion of the food substance within the tube; that the form of the curve describing growth is wholly dependent upon the course of the reaction involved in the digestion of the reserve food substance, is

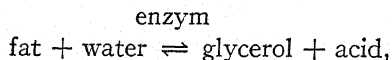


less certain. That pollen-tube growth is an autocatalytic process dependent directly on the hydrolytic and synthetic reactions involved in the production of food material in the tube, however, is a simple and attractive explanation of the phenomenon. Let us see how it fits the known facts regarding pollen-tube growth on artificial media and in the style of the plant.

In monomolecular autocatalytic reactions, it is assumed that one large molecule, the substrate, is undergoing a process of being divided up into smaller ones under the action of a catalyst. In the case of pollen-tube growth the substrate is qualitatively the same throughout the whole growth cycle, and its nature is revealed by the character of the reserve food materials in the pollen grain. The catalyst is an appropriate enzyme. In the form used we are dealing with a fat $\rightleftharpoons$ glycerol + fatty-acid system, and the reaction would be represented thus:



This is, of course, a reversible reaction, the enzyme accelerating it in both directions. Now, if we consider the equilibrium positions of this system under different conditions, we find a very plausible explanation of the facts at hand regarding pollen-tube growth. The knowledge that reactions in the living cell are reversible presupposes a mechanism for controlling the amount of water in the system. If this were not so, all reactions could go in but one direction. In pollen-tube growth *in vivo*, the reserve material, fat or starch as the case may be, hydrolyzed in the process is replaced directly or indirectly from sugar derived from the stylar tissue. Growth is a continuous process of hydrolysis and synthesis of fat in the tube, the rates being determined by the masses of the reacting substances. If the mass of the water component of the system were low, the reverse reaction could proceed as rapidly as hydrolysis. Under these conditions the effect due to a deficiency of substrate would not be observed, and we should get a growth curve such as East and Park (1918) found for pollen tubes in the styles of *Nicotiana*. On the other hand, on the artificial cultures the pollen tubes are growing in a watery medium. Owing to the excess of water present, the equilibrium position in the system:



is very near complete hydrolysis. The amount of synthesis is too small to be perceptible; that is, the reaction goes almost entirely to the right and terminates, as does growth, when the substrate is exhausted, giving an S-shaped curve.

Thus far we have not considered the influence of the sugar present in the artificial medium. It has already been noted that there is a more or less general tendency toward asymmetry in the curves and that the maximum



rate of growth especially in *Scilla* does not occur when growth is just half completed. It seems to come somewhat earlier in the cycle, and the curve in its later stages shows a slowly decreasing growth rate. The simplest interpretation of this asymmetry is that the sugar supplied in the artificial medium supplements the food materials formed by hydrolysis of the reserves and thus prolongs growth especially in the later stages. If the sugar is actually a component of the reaction system, such an effect would result from its mass action. The position of equilibrium, however, would not be materially altered, because of the great preponderance of water in the system.

Green (1894), working with lily pollen, has demonstrated a rapid increase in the quantity of enzym present during germination and the early stages of growth. It may well be that this is the cause of the increasing rate of growth at this stage. There are reasons other than exhaustion of the reserve food material in the pollen tube, however, that might account for the decline in growth in the later stages. Among these we may mention two: (1) exosmosis of some essential constituent of the cell; and (2) accumulation of products of metabolism having a toxic effect.

The hypothesis that pollen-tube growth on artificial media is an autocatalytic reaction in which the substrate is limited seems to interpret in a plausible manner the important difference (if we may generalize on the one hand from the single but carefully analyzed case of East and Park (1918) on pollen-tube growth in the style of *Nicotiana*) which exists between pollen-tube growth *in vitro* and *in vivo*. If this difference is dependent, as our hypothesis holds, upon the difference in the water relations in the two cases, we should be able by varying this factor in the artificial cultures to change the shape of the growth curve. Only on artificial media containing a minimum of water could we expect to get a growth curve similar to that found by East and Park. Definite conclusion as to the adequacy of our hypothesis to interpret pollen-tube growth must await the results of such experiments.

#### EXPLANATION OF PLATES

##### PLATE XIX

Distribution of the nuclei in the pollen tubes of *Scilla* grown on a medium consisting of 2 percent agar, 7 percent cane sugar, and a little sterile yeast. Figures drawn from whole tubes mounted in aceto-carmine. The drawings in part were made with the aid of the camera lucida; the remainder were sketched free-hand.

FIG. 1. Pollen grain in the resting condition, showing a single nucleus.  $\times 400$ .

FIG. 2. Pollen grain in the resting condition, showing two nuclei.  $\times 400$ .

FIGS. 3, 4. Germination at 15 minutes.  $\times 400$ .

FIG. 5. Migration of nuclei from the pollen grain at 1 hour.  $\times 400$ .

FIG. 6. Position of the nuclei at  $1\frac{1}{2}$  hours.  $\times 300$ .

FIG. 7. Tip of fully grown tube at 5 hours, showing the two nuclei in the tip.  $\times 300$ .

FIG. 8. Tip of tube 6 hours old, showing the tube nucleus and two sperm nuclei.  $\times 300$ .

- FIG. 9. Tube nucleus and two sperm nuclei at 7 hours in the tip of a long tube.  
× 300.  
FIG. 10. Tube 1.37 mm. long at 6 hours, showing aberrant distribution of the nuclei.  
× 300.  
FIG. 11. Tube 1.43 mm. long at 6 hours, showing unusual distribution of nuclei.  
× 150.  
FIG. 12. Pollen tubes removed from the style at 1 hour.

## PLATE XX

Figures 13-16 were drawn from pollen tubes of *Vinca minor* growing on an artificial medium. Figures 17-23, showing the digestion of the reserve fatty material, were drawn from pollen tubes of *Vinca minor* grown in a medium composed of 2 percent agar, 10 percent cane sugar, and a little sterile yeast. The tubes were mounted in Sudan III and sketched free-hand. Magnification, 200 diameters.

FIGS. 13-16. Four stages in the development of callose plugs.

FIG. 17. Pollen tube from a culture 1 hour old, or about 15 minutes after germination.

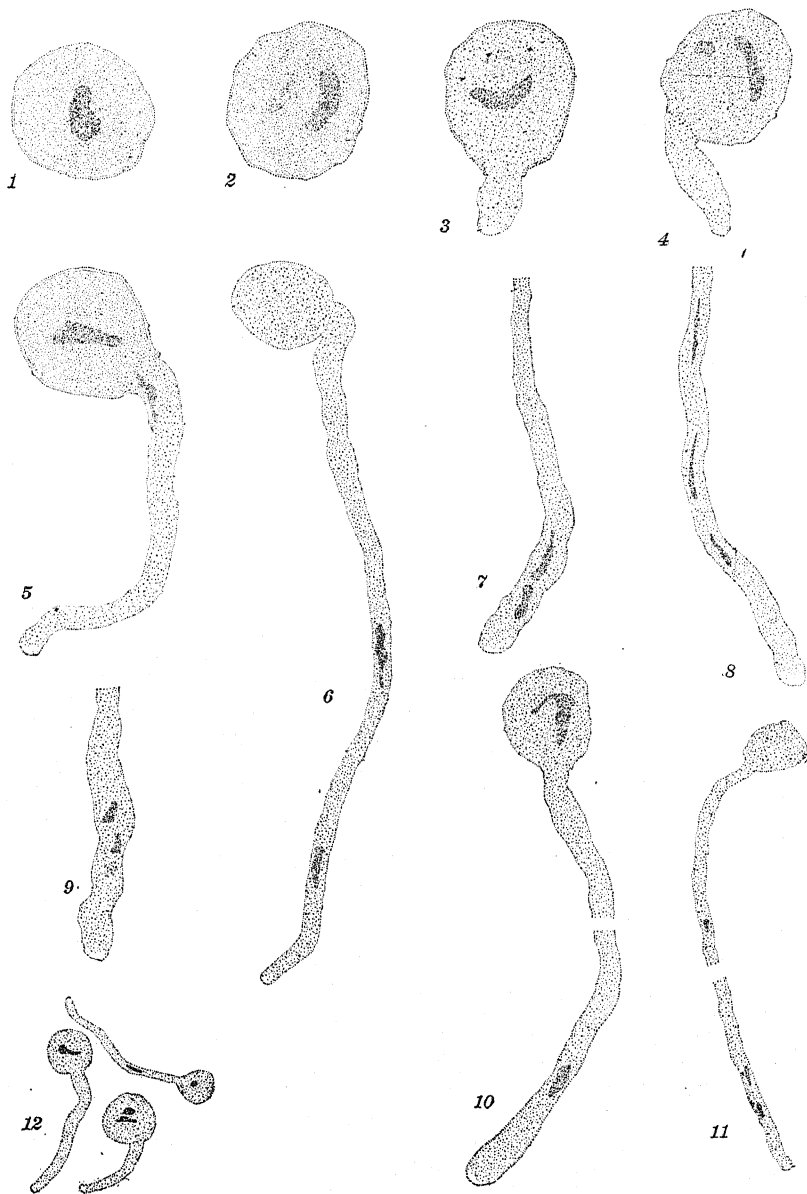
FIG. 18. Pollen tube from a culture 1½ hours old.

FIGS. 19, 20. Tips of pollen tubes at 3 hours.

FIGS. 21, 22. Tips of long pollen tubes from culture 6 hours old, showing advanced stages in the digestion of the reserve food material.

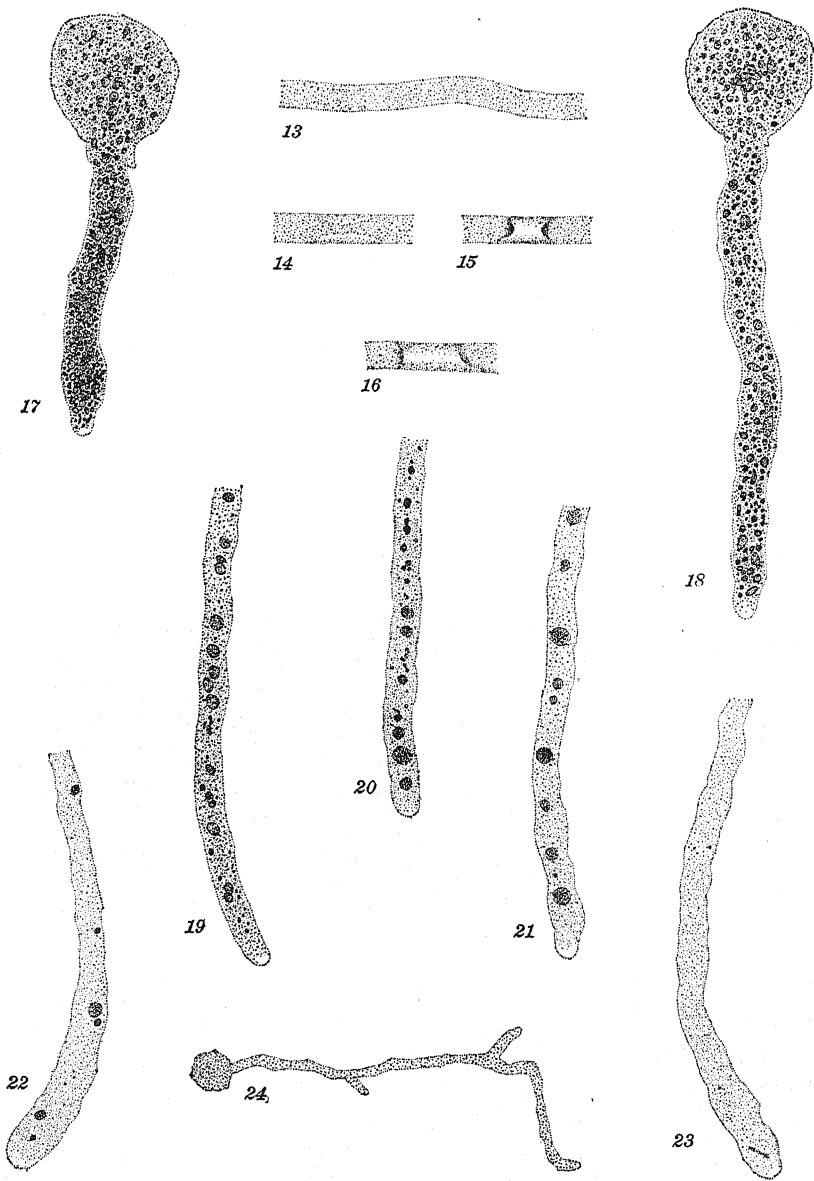
FIG. 23. Tip of long pollen tube at 6 hours, in which the reserve material has been entirely digested. A single nucleus is shown in the tip.

FIG. 24. Pollen tube of *Cucurbita pepo* grown *in vitro*, showing the branching habit.  
× 40.



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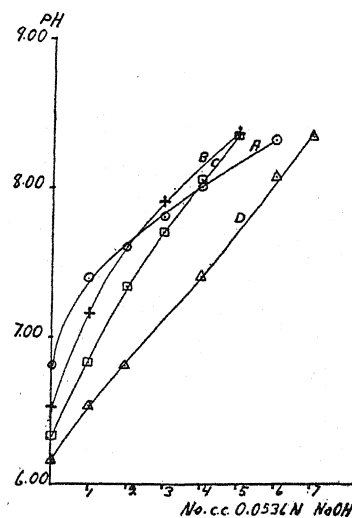


# TOTAL ACIDITY COMPARED WITH ACTUAL ACIDITY OF PLANT JUICES

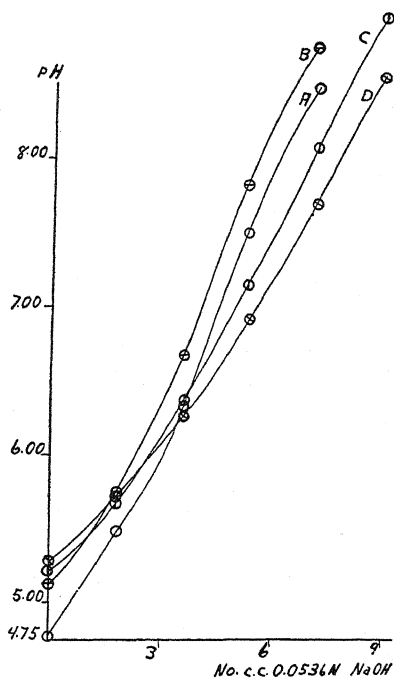
FELIX G. GUSTAFSON

(Received for publication August 10, 1923)

In a recent paper <sup>1</sup> the writer has pointed out that in many plants there is a hydrogen-ion concentration gradient. As stated in the above-mentioned paper, the gradient is not due to unequal dilution of the cell contents nor to unequal amount of CO<sub>2</sub> dissolved in the juice. It might be due to a difference in total acidity in different parts, to an unequal distribution of basic elements, or even to differences in rate of respiration. This work is an attempt to discover whether there is any correlation between total acidity and H-ion concentration.



TEXT FIG. 2. Titration curves of the juice from the leaves of *Helianthus* sp. Curve A represents the titration of the leaves from the first node from the base, B that of the leaves from the second node, C of the leaves from the third node, and D of the leaves from the fourth node.

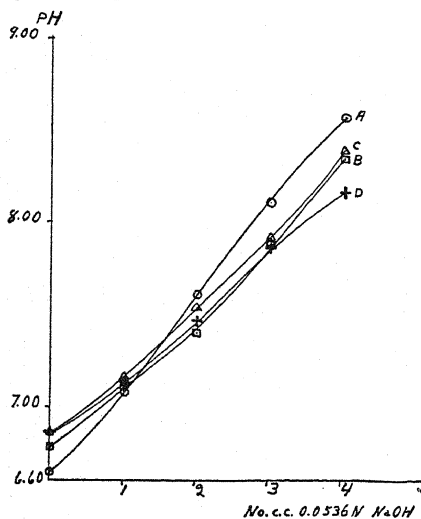


TEXT FIG. 1. Titration curves of the juice from *Zea mais* leaves. Curve A represents the titration of leaves from the first node from the base of the plant, B of the leaves from the third node, C of the leaves from the fourth node, and D of the leaves from the eighth node.

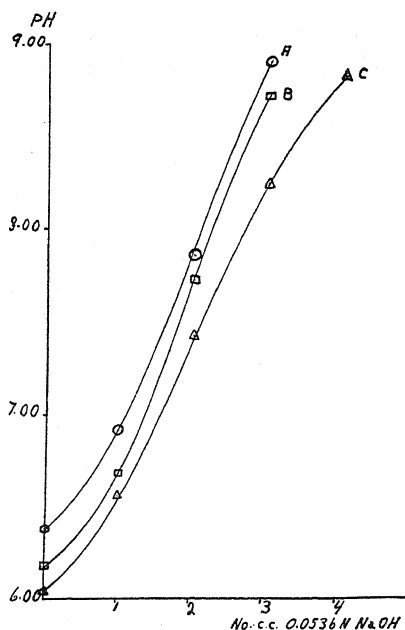
<sup>1</sup>Gustafson, F. G. Hydrogen-ion concentration gradient in plants. Amer. Jour. Bot. 11: 1-6. 1924.



The total acidity was determined by electric titration. Samples of 10 cc. were used for each determination. The pH of each sample was determined before the addition of any hydroxid, and also after each addition of 1 cc. of NaOH. Using these readings as points, curves were constructed illustrating the trend of neutralization as well as giving the amount of hydroxid required to neutralize the acid substances in the juice. It will be noticed by reference to the graphs that there is considerable variation in the character of the titration curves from different plants. For *Zea mais*, for instance, the curves are steep, while for *Bryophyllum calycinum* they are not nearly as steep.



TEXT FIG. 3. Titration curves of juice from the leaves of *Cucurbita maxima*. Curve A represents the titration of leaves from nodes one and two, B of the leaves from nodes three and four, C of the leaves from nodes five, six, and seven, D of the leaves from nodes eight, nine, ten, and eleven.

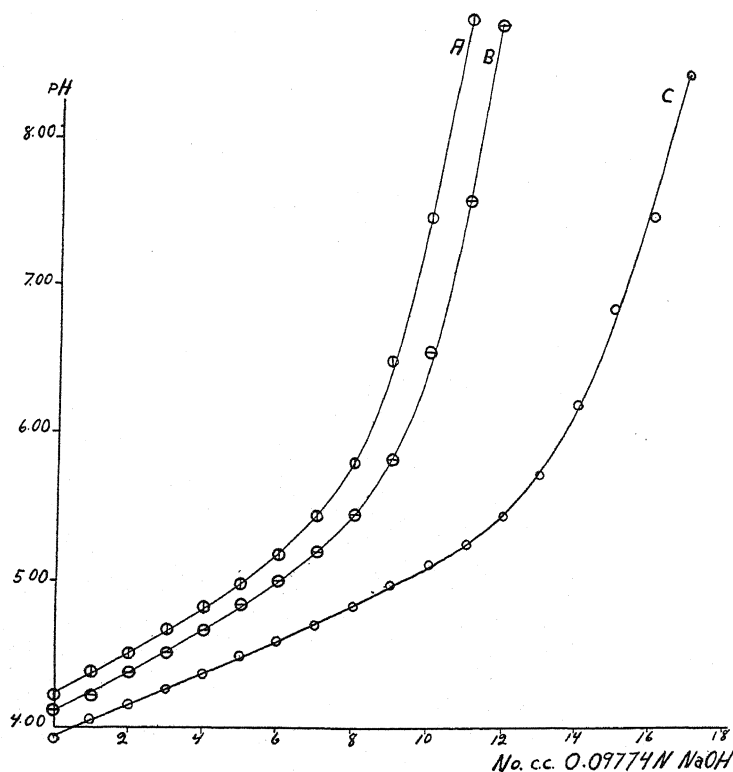


TEXT FIG. 4. Titration curves of the juice from the stem of *Cucurbita maxima*. Curve A represents the titration of the juice from the first 10 cm. of the stem from the base, B of that from the next 18 cm., and C from the remainder of the stem.

The *Zea mais* plants were used at the time of tasseling, each having at that time about ten leaves all in good condition. The plants were grown in the greenhouse and were not as tall and robust as when grown out of doors, yet they were healthy plants. There was the usual H-ion concentration gradient, but there was no relation between the original pH of a sample of juice and the amount of hydroxid required to bring it to pH 8.3, which was chosen as the end point. The leaves from the eighth node had originally the highest pH (5.27) and were already nearest the end point; yet, to reach that end point, 9 cc. of NaOH were required, as compared with 7 cc. for the leaves from the first node, which at the beginning was

farthest (pH 4.75) from the end point. In other words, 7 cc. of hydroxid were required to bring about a change from pH 4.75 to 8.3, while 9 cc. were required to change the reaction of the juice from the youngest leaves from pH 5.27 to 8.3. Only 6.2 cc. of hydroxid were needed to neutralize 10 cc. of the juice from the third node; its original pH was 5.13. From these data it will be seen that there is no relation between the original pH of the juice of corn leaves and their total acid content.

Leaves of *Helianthus* sp. and of *Cucurbita maxima* are similar to those of *Zea mais* in that there is no relation between pH and total acidity. In the



TEXT FIG. 5. Titration curves of the juice from the leaves of *Bryophyllum calycinum*, on a cloudy day. Curve A represents the titration of the leaves from node three, B from node four, and C of the leaves from nodes eight and nine.

stem of *Cucurbita maxima* there is a relation between total acidity and pH. That part of the stem having the highest pH has the lowest total acid content, and that part having the lowest pH has the highest total acid content.

Determinations made on the juice from the leaves of *Bryophyllum calycinum* during a cloudy day show a relation between pH and total acidity. In all instances, as the H-ion concentration increases the total acidity also increases. On the other hand, when determinations were

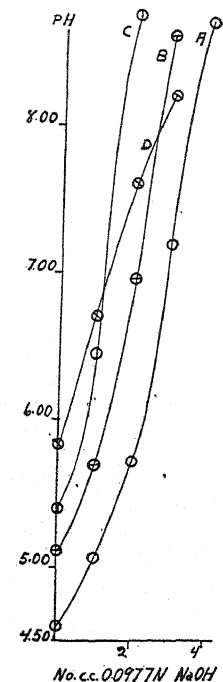
made on a clear and sunny day, there was no relation between H-ion concentration and total acid.

Another interesting fact was brought out by the experiments on *Bryophyllum calycinum*; in the first experiments the oldest leaves had the highest pH, while the youngest had the lowest pH. In the experiments conducted during a sunny day there is a gradient of H-ion concentration,

but it is the reverse of that found during the cloudy day, *i.e.*, the oldest leaves now have the lowest pH and the youngest leaves have the highest.

As shown by the graphs, there is a difference, both in H-ion concentration and in total acidity, in leaves of *B. calycinum* on a cloudy day and on a bright day. The fluctuation of acidity is in agreement with the findings of Heyne<sup>2</sup> who in 1813 wrote that in the morning the leaves of *Bryophyllum* (*Cotyledon*) *calycinum* are as acid to the taste as sorrel, while, as the day advances (as the light becomes more intense), they lose their acidity, being tasteless by noon. Kraus<sup>3</sup> corroborated Heyne's observations, by testing the juice from leaves of *B. calycinum* with litmus paper. As far as the writer is aware, the fluctuation in H-ion concentration has not been reported before. The greatest fluctuation is found in the youngest leaves, where the metabolism is presumably most active, and the least in the oldest leaves.

It is worth noting that the juice from the youngest parts of all plants used, stems and leaves, requires more NaOH to neutralize it than the juice from the oldest part, even when the pH of the younger part is higher at the beginning, *i.e.*, already nearer the end point. This would seem to indicate that the young portions of plants contain (at least in some instances) acid substances that do not dissociate as highly as those found in older parts, because, though the juice contains much total acid, yet it has not a high concentration of hydrogen ions, and a great percentage of the acid is undissociated; or the dissociation may be the same, but, the juice of the younger parts being more viscous, there is a possibility that there is a greater



TEXT FIG. 6. Titration curves of the juice from the leaves of *Bryophyllum calycinum* on a sunny day. Curve A represents the titration of leaves from node one, B of the leaves from node two, C of the leaves from node three, and D of the leaves from node eight.

adsorption of the H ions, which on neutralization would separate from the colloidal material of the juice and then be neutralized. There is a third con-

<sup>2</sup> Heyne, B. On the deoxidation of the leaves of *Cotyledon calycinum*. Trans. Linn. Soc. 11: 213. 1814.

<sup>3</sup> Kraus, G. Ueber die Wasservertheilung in der Pflanze IV. Die Acidität des Zellsaftes. Abhandl. Naturf. Ges. Halle 16: 154. 1883.

tingency, namely, the greater adsorption of the hydroxyl ions by the colloids of the younger leaves.

#### SUMMARY

As illustrated by *Zea mais*, *Cucurbita maxima*, *Helianthus* sp., and *Bryophyllum calycinum*, the total acid of the plant juice is not responsible for the H-ion concentration gradient found in plants. There is no constant relation between total and actual acidity in these plants.

The juice from young parts of plants requires more NaOH to neutralize it than does juice from older parts of the same plant, even when the former is nearer to the neutral point at the beginning than the latter.

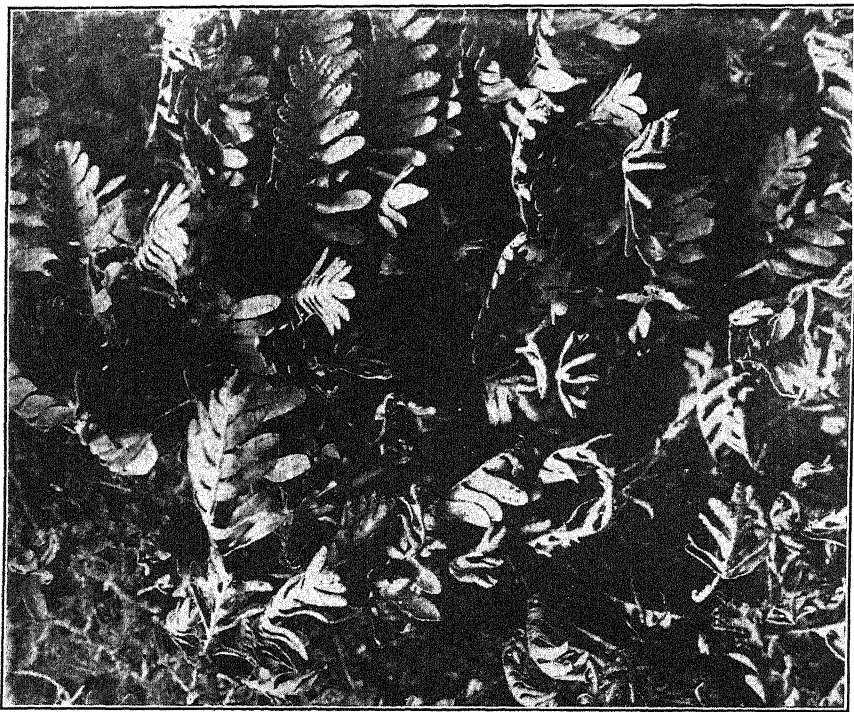
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A PHYSIOLOGICAL AND ANATOMICAL STUDY OF THE  
LEAVES OF *POLYPODIUM POLYPODIOIDES*<sup>1</sup>

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While studying the effect of the climatic conditions on the distribution of *Polypodium polypodioides* (L.) Hitchc., the writer observed a peculiar curling of the leaves of the fern under dry conditions. This characteristic behavior of the leaves of the epiphytic polypody in dry weather seems at



TEXT FIG. 1. An expanded mass of *Polypodium polypodioides*.

first sight to serve as a special and adequate adaptation against desiccation (compare text figs. 1 and 2).

The curling on drying begins, as a rule, by the rolling inward of the pinnae from the ends with the upper surface inward. As the entire leaf is folding, each individual pinna also folds with its upper surface inward so that there is really a double folding in each pinna, one main rolling-in

<sup>1</sup> Botanical contribution from the Johns Hopkins University, no. 77.

endwise of each pinna towards the rachis and another curling upward and inward of the margins of each pinna towards its midrib. The folding of the individual pinnae is soon followed by the curling of the leaf, and while this takes place the rachis begins to twist and to curl. Thus, in all cases, the curling of the leaf causes the upper epidermis to be hidden from the outside, while the lower epidermis, which is covered with numerous scales, remains exposed. This characteristic curling, which conceals the upper surface and leaves only the lower surface exposed, suggests at once an adaptation of the leaf to xerophytic conditions. It might be assumed from such a behavior



TEXT FIG. 2. The same mass of the polypody artificially dried, showing the characteristic curling of the leaves.

of the leaf that its upper epidermis loses water more rapidly than its lower one, and that, by being rolled in when the air is dry, the leaf avoids the danger of excessive transpiration from the upper surface. The scales which cover the lower surface of the leaf might well be assumed to function as an aid in lessening the evaporation from the lower epidermis. To determine definitely whether these assumptions are correct, and to learn which side of the leaf really does lose the greater amount of water under given conditions, experiments were carried on in the laboratory both on living and on dead leaves of the epiphytic fern. Abundant material for these experi-



ments was obtained from Mississippi through the kindness of Professor J. M. Beal, and a large clump of the living fern was sent from Athens, Georgia, by Professor J. M. Reade. To both of these gentlemen the writer here expresses his sincere thanks.

#### METHODS OF EXPERIMENTATION

Three experiments were performed on detached leaves of *Polypodium polypodioides*. In the first experiment, four groups of four leaves each were placed in a desiccator containing a uniform layer of calcium chlorid. In the first group, each leaf received a thin layer of petrolatum on both the upper and lower epidermis, thus sealing up both surfaces completely. The leaves of the second group received a layer of petrolatum on the upper surface only, leaving the lower one open; those of the third group were sealed only on the lower side, leaving the upper side untouched; but the leaves of the fourth group were left in the natural condition so that transpiration could take place normally from both the upper and the lower surfaces. In each case only the leaf blade was used, in order to avoid any complications arising from an uneven sealing of the cylindrical petiole. After the sealing, each leaf was weighed, then dried in the desiccator for twenty-four hours and weighed at the end of that time. The weighing was repeated daily for one week. At the end of this experiment, the recorded weights of each group were averaged and the percentage of water lost from each surface was calculated from the results obtained. Having thus determined the rate of water loss from each surface of the leaves, these same leaves were then suspended from wires in a moist chamber so that each leaf was a centimeter or so above the water level but not actually in contact with the water. This was done in order to determine the water-absorbing power of each surface of the leaf. As before, the leaves were weighed daily for one week. At the end of this test, the average percentage of gain from each group was calculated.

In the second experiment the method was somewhat modified. Instead of using calcium chlorid as the drying agent, sulfuric acid was used, since by means of this acid it was possible to establish different degrees of drying. Forty cubic centimeters of concentrated sulfuric acid (100 percent) constituted the drying agent in the first Mason jar which was sealed tightly by a paraffined cork stopper. From the inner side of the cork, eight leaf-blades were suspended from hooks fastened in the cork. Of the eight leaves, two were vaselined on both sides, two on the upper side only, two on the lower side only, and two remained uncoated for controls. In no case were the leaves allowed to be close to the acid; they were generally about six centimeters above its surface. The leaves in the second jar were similarly arranged, but this jar contained forty cubic centimeters of dilute sulfuric acid in the proportion of one part of water to three parts of acid (75 percent). A third jar contained a like quantity of half water and half acid (50 percent);



a fourth contained one part of acid to three parts of water (25 percent); a fifth, one part of acid to nine parts of water (10 percent); and a sixth jar contained only distilled water (00 percent). Thus, each jar contained a series of leaves some with the upper surface free; some with the lower surface uncoated; some with both upper and lower surfaces clear and some with both surfaces sealed by the vaseline. It may be mentioned here that white vaseline was used in preference to cocoa butter since the latter tends to harden, crumbles, and drops off when the leaf begins to curl in the desiccator, thus introducing an error when the leaves are weighed. All leaves of each series, being in the same jar, were exposed to precisely the same drying conditions. The very same experiment was also made with leaves which had been previously killed by being subjected to chloroform vapor for three hours, after which time they became brown and began to curl. Thus, both living and killed leaves were subjected to similar drying conditions. Each leaf from every desiccator was weighed once in twenty-four hours for one week.

The third experiment was designed to determine any internal structural changes in the leaf when in either the dry (curled) condition or in the moist (expanded) condition. Small pieces were cut from curled and expanded leaves of plants grown in the open. These were immediately dropped into absolute alcohol and kept there for thirty-six hours, the alcohol being frequently changed to insure complete dehydration. The material was thus fixed before any internal changes could take place. Later, the ordinary method was used in imbedding and sectioning this fixed material. For fixing the sections on the slide no water was used, nor was there any water (or dilute alcohol) used in the staining of the preparations, this being done in absolute alcohol entirely. Throughout this procedure the leaves retained their original shapes as cut in the field, and the sections cut from them showed the difference between the internal structure of the curled leaf and that of the expanded one.

#### RESULTS

The results of the first experiment show that normal unsealed leaves lost on an average of 68.13 percent of their weight during the first twenty-four hours; during the second day the loss amounted to 8.75 percent of their weight; while for the rest of the week these leaves lost no more in weight. The total average loss from the uncoated leaves for the entire week amounted to 76.88 percent of their original weight. The one externally evident result of this enormous loss was the curling of the leaf. The leaves of which the lower surfaces were sealed showed an average loss during the first day of 11.16 percent, during the second 5.12 percent, during the third 8.72 percent, during the fourth 0.32 percent, and during the fifth 1.07 percent. No more loss in weight took place during the rest of the week. Those leaves of which the upper surfaces were sealed lost during the first day an average of 42.30

percent, during the second 3.67 percent, and during the third day 7.4 percent. No further loss was later observed. The leaves with both surfaces sealed lost the least, the first day's loss being 6.04 percent. During the second day the loss was 1.33 percent, and on the fourth day a loss of 4.82 percent was observed. These leaves showed no further loss. Theoretically these leaves should lose no water at all; but, since a complete sealing of the leaf is practically impossible, it may be assumed that the recorded loss took place from the unsealed patches on the edges of the leaf. At any rate, the result of prime interest here is that the leaves lost most water when both surfaces were open. From the lower surface alone the leaves lost 53.18 percent during the entire week; while from the upper surface alone the leaf lost only a total of 26.39 percent during the whole week. It is evident from these results that the leaves of this fern lose water almost twice as rapidly from their lower surfaces as from their upper ones. This seems rather puzzling, for it is actually the lower side that is exposed when the leaf curls up in dry weather, while the side losing the least water is concealed and subject to very little desiccation.

The results obtained from the second experiment are indicated in tables 1 and 2. A study of the data shows that, when the leaves were subjected

TABLE 1. *Living Leaves in Sulfuric-Acid Desiccator Tested for their Capacity to Lose Water*

Series*	Surface Sealed	Percent Loss in Weight during Following Days							Total Loss Percent
		1st	2d	3d	4th	5th	6th	7th	
I 100% acid	None	57.4	.....	.....	.....	.....	.....	.....	57.4
	Upper	36.2	2.0	4.0	.....	.....	.....	.....	42.2
	Lower	20.5	10.0	6.6	.....	.....	.....	.....	37.1
	Both	6.0	1.1	.....	.....	.....	.....	.....	7.1
II 75% acid	None	45.2	9.8	.....	.....	.....	.....	.....	55.0
	Upper	36.6	4.7	2.3	.....	.....	.....	.....	43.6
	Lower	11.5	3.3	6.9	.....	.....	.....	.....	21.7
	Both	7.3	.....	.....	.....	.....	.....	.....	7.3
III 50% acid	None	55.5	9.5	.....	.....	.....	.....	.....	65.0
	Upper	54.4	.....	.....	.....	.....	.....	.....	54.4
	Lower	21.3	.....	.....	.....	.....	.....	.....	21.3
	Both	6.2	2.3	.....	.....	.....	.....	.....	8.5
IV 25% acid	None	50.9	.....	.....	.....	.....	.....	.....	50.9
	Upper	27.6	5.3	7.3	.....	.....	.....	.....	40.2
	Lower	10.1	.....	2.5	.....	.....	.....	.....	12.6
	Both	7.2	.....	.....	.....	.....	.....	.....	7.2

to artificial drying in the sulfuric-acid desiccator, they behaved essentially as they did in the calcium-chlorid desiccator. In both cases the greatest amount of water was lost from the lower surface of the leaf. This was true, not only for living leaves, but likewise for leaves which had been killed by chloroform. It is safe to conclude from these results that the lower surface,

although covered with scales and apparently resistant to drought, is really the surface which loses water most rapidly. It is needless to say that, when an appreciable amount of water is lost, a folding or a curling of the leaf results. In both living and dead leaves, those of which neither surface was sealed curled up completely when subjected to dry conditions; those of which only the lower side was sealed curled not quite as markedly as in the former case; the leaves of which the upper side only was sealed became partially folded; while those with both surfaces sealed remained completely expanded throughout the experiment. The condition of the living leaves remained

TABLE 2. *Leaves Killed in Chloroform and Placed in Sulfuric-Acid Desiccator to Test their Capacity to Lose Water*

Series*	Surface Sealed	Percent Loss in Weight during Following Days							Total Loss Percent
		1st	2d	3d	4th	5th	6th	7th	
I 100% acid	None	52.9	3.3	.....	.....	.....	.....	.....	56.2
	Upper	35.3	.....	.....	.....	.....	.....	.....	35.3
	Lower	17.5	8.2	.....	.....	.....	.....	.....	25.7
	Both	11.4	8.4	.....	.....	.....	.....	.....	19.8
II 75% acid	None	44.5	.....	.....	.....	.....	.....	.....	44.5
	Upper	41.1	.....	.....	.....	.....	.....	.....	41.1
	Lower	26.2	.....	.....	.....	.....	.....	.....	26.2
	Both	11.1	.....	.....	.....	.....	.....	.....	11.1
III 50% acid	None	40.5	.....	.....	.....	.....	.....	.....	40.5
	Upper	32.5	.....	.....	.....	.....	.....	.....	32.5
	Lower	18.9	.....	.....	.....	.....	.....	.....	18.9
	Both	9.3	.....	.....	.....	.....	.....	.....	9.3

\* In the series containing 25 percent acid, the killed leaves showed no decrease in weight. In the desiccators containing 10 percent acid or distilled water, neither the living nor the killed leaves showed any decrease in weight.

unchanged in the jar containing 10 percent acid and in the one containing distilled water, indicating that there was no loss of water from the leaves in that atmosphere. The killed leaves, however, failed to show any loss in weight even in the jar containing 25 percent acid. Furthermore, these soon became infected by a fungus in the jars containing 25 percent and 10 percent acid and in those containing distilled water.

The results of the experiments on the water-losing power of the leaves, which show that the lower surface loses water about twice as rapidly as the upper one, discredit at once the idea that the protection of the upper surface by curling is a very effective adaptation against excessive transpiration. The rolling inward of the upper surface does, of course, reduce the transpiring area in half, but it leaves the more active half, which contains all the stomata, still exposed. The question, then, arises whether the lower surface may not really be especially capable of water-absorption. To determine the water-absorbing power of each surface of the leaf, dried curled

leaves were placed in a moist chamber. When those unsealed leaves which had been kept in the desiccator, and which were completely curled after drying, were later placed in the moist chamber, they gained 44.53 percent of their weight during the first twenty-four hours and became fully expanded. During the second day the gain was 4.84 percent, during the third 4.58 percent, during the fifth 6.15 percent, and during the sixth and seventh days the leaves showed no gain in weight. The average total gain for the whole week amounted to 60.10 percent. Those leaves whose upper surface only was sealed gained during the first day 16.29 percent, during the second 3.45 percent, during the third 7.8 percent, during the fourth 1.82 percent, during the fifth 0.63 percent, and during the sixth and seventh days together the gain observed was only 0.62 percent, making a total of 30.61 percent. At the end of the fifth day these leaves began to unfold, and by the end of the sixth day they were fully expanded. The leaves whose lower surfaces were sealed showed a gain of only 1.25 percent during the first day, of 1.64 percent during the second day, of 0.52 percent during the fourth, and of 0.01 percent during the fifth and sixth days together, making a total for the whole week of only 3.42 percent. This gain in weight was accompanied by no appreciable change in the physical appearance of the leaf. Whether any part of the observed gain in these three experiments was due to the actual condensation of the water vapor present about it by the leaf itself is an unsettled question. It is certain that no leaf actually came in contact with the liquid water which covered the floor of the chamber. It is quite possible, however, that during the night the water vapor was condensed on the surface of the leaves, so that the leaves may have obtained the water entirely by the imbibition of the liquid water condensed on their surfaces and not by condensing the water vapor that penetrated to the interior by way of the stomata. At any rate, it is noteworthy that the leaf increased in weight nearly ten times as rapidly when the lower surface was uncoated as when the upper one was left free. Whether this enormous increase in weight is due to the fact that all the stomata of the leaf are found on its under side and none on the upper side, thus offering more absorbing surface, is a question that needs further investigation.

The difference in the appearance of the leaf under wet and dry conditions suggests that there may be internal structural differences between the expanded form and the curled one. Paraffin sections were made of both curled and expanded leaves fixed in absolute alcohol. These showed a remarkable difference in the tissues of the leaf. The cells of the expanded leaf seemed to be turgid and maintained their maximum size and smooth outline. The cells of the curled leaf, on the contrary, were greatly reduced in size and wrinkled in outline. The shape of a cross section of a curled leaf is shown in Plate XXI, *G*. The diagrammatic drawings in Plate XXI, *I* and *H*, show the comparative size and shape of the cells from cross sections respectively of a curled and of an expanded leaf. Both of these sections

come from the same region of corresponding pinnae, and the same magnification is used for both.

Whether the loss and gain of water by the leaf is entirely an osmotic phenomenon or is accomplished partly or entirely by imbibition, might be answered by a measurement of the cells of the leaf in the curled condition and of those of the leaf in the expanded condition. Paraffin sections of curled leaves were mounted on slides, the paraffin was dissolved off by xylol, and the preparation was covered with a cover slip. In this manner, while the sections were still in xylol under the cover glass, individual cells picked at random in three different regions of the upper and lower epidermis and in the palisade tissue were measured in width and length. The two dimensions of a number of cells of each region of a given tissue were then averaged, and these were regarded as the dimensions of a typical cell of the curled leaf. After the measurements were obtained, the xylol was drawn off from beneath the cover slip and was replaced by absolute alcohol while the sections were still under observation under the microscope. When all the xylol was drawn out, the alcohol was drawn off in the same manner and water was introduced to replace the alcohol, its effect on the cross section of the curled leaf being noted while the water was spreading under the cover glass. As soon as the water came in contact with the cell walls of the cells in the section, the cells began to swell and to expand so that at first a vertical straightening of both ends took place; shortly after, the midrib region expanded, and, in straightening, forced the two ends in opposite directions, bringing about thus the expanded shape of the normal leaf under moist conditions. By this means it was possible actually to measure the cells in the curled state, and again those of the same leaf in the expanded state. When the sections were fully expanded, a number of cells in each of the three different tissue layers in the different regions of the leaf were again measured, precisely as in the case of the curled sections. The results obtained from measuring the dimensions of the cells in both conditions are given in table 3. It is evident from the data that all the cells of the leaf do not increase in size equally when water is introduced. The cells of the upper epidermis above the midrib increase about twice as much in width as do those of the epidermis on the lower side of the midrib. The increase of the cells near the margin in the upper epidermis is slightly less than in the lower epidermis; while the epidermal cells in the region between the margin and the midrib of both the upper and the lower sides show a considerable increase, although the cells of the upper side increased about a third more than those of the lower side. It will be noted that the greater increase takes place in the width of the cells of both of these tissues; the increase in length is decidedly smaller, and is practically alike in both tissues. In the palisade tissue, the greatest increase in length and width occurs in the region between the midrib and the margin, while the smallest increase occurs at the margin.

TABLE 3. Increase in Size of Cells in a Cross Section of a Curled Leaf after Wetting

Condition of Leaf	Tissue	Region in Leaf	Average		Percent Increase	
			Width, $\mu$	Length, $\mu$	Width	Length
Curled (before wetting)	Upper epidermis	Midrib	7.5	14.6		
		Margin	10.5	16.3		
		Intermediate	7.8	15.8		
Expanded (after wetting)	Upper epidermis	Midrib	19.3	17.8	157.3	21.9
		Margin	18.7	17.5	78.6	4.5
		Intermediate	18.9	18.7	143.8	18.7
Curled (before wetting)	Lower epidermis	Midrib	10.5	12.3		
		Margin	10.7	12.2		
		Intermediate	11.0	12.4		
Expanded (after wetting)	Lower epidermis	Midrib	18.7	15.2	82.0	23.6
		Margin	20.6	17.1	92.5	40.1
		Intermediate	22.7	14.8	106.3	19.44
Curled (before wetting)	Palisade	Midrib	5.0	23.8		
		Margin	6.2	22.0		
		Intermediate	5.0	25.0		
Expanded (after wetting)	Palisade	Midrib	13.0	36.4	106.0	52.8
		Margin	12.5	28.7	101.5	30.5
		Intermediate	15.0	40.0	200.0	60.0

If we regard the increase in size of the cells after the introduction of water as an index of their capacity to lose water during drying, it becomes evident that the cells of the upper epidermis at the leaf margin must lose least water, while those of the midrib region must lose most. The lower epidermal cells of the midrib region, however, lose less water than the upper epidermal cells of the same region. It is further evident that the greatest decrease in size, under dry conditions, in all the cells measured is that in width. It thus appears that the curling of the leaf is caused chiefly by the unequal rate of water-loss of the cells of the different parts of the leaf.

#### PHYSIOLOGICAL SIGNIFICANCE OF THE SCALES

The presence of the scales on the lower surface of the polypody suggests their possible function as a protection against desiccation. According to Karsten (2), the scales present on the under side of the stem of *Polypodium imbricatum* serve to distribute the water drops which settle on the stem. In *Polypodium sinuosum*, however, Karsten says that the scales serve for absorbing water which appears on the stem in the form of drops. Both Schimper (4) and Mez (3) attribute the function of water-absorption in *Tillandsia* to the scales. Mez regards the mechanism by which the water is absorbed by the scale as either osmotic or capillary. So far as the writer knows, the function of the scales in *Polypodium polypodioides* has not been determined. Although in a number of sections of dry leaves the wing of the



scale was pressing closely against the surface of the leaf, other sections, on the contrary, seemed to show that the scale stood up above the surface so that there was a space between the surface of the leaf and the wing of the scale. A similar behavior of the scales was also observed in sections of the expanded leaf. It is therefore difficult to ascribe any definite function to the scales. There are some indications that they aid in the absorption of water, for the thick-walled cells of the wing were seen to absorb water rapidly. Whether the water absorbed by these thick cell walls is later carried over by capillarity to the living stalk cells and from there to the interior of the leaf, the writer is unable to say with any degree of definiteness. It is also quite possible that they help to protect the plant against drought. Their primary function seems to be, however, that of holding a film of water on the under surface of the leaf long enough to allow this water to be imbibed by the cell walls, in this way helping to restore the leaf to the expanded form. This is particularly plausible when one considers the short time it takes a dry and curled leaf to expand after a rain. Long before the roots within the bark are able to take up and supply to the leaves the water absorbed by the bark during the rain, the surface cells of the leaf evidently absorb water and begin to enlarge, and thus begin the uncurling of the leaf.

#### LEAF-MOVEMENT

Harshberger (1) who studied the curling of the leaves of *Rhododendron maximum*, in which it is the upper instead of the lower epidermis that becomes exposed during curling, claims that the rolling movement of the leaf is due to the gradual passage of the sap into the intercellular spaces, or due "to the movement of the liquid from cell to cell by means of protoplasmic bridges so that one part of the leaf becomes highly turgescient and the other part more or less flaccid." He also believes that low temperature is responsible for the moving of the liquids towards the upper side of the petiole and of the leaf; while a higher temperature reverses the process. Harshberger seems to think that turgidity is the main factor in the mechanism of the leaf movement.

It was found from a study of cross sections of a curled leaf that the cells showed considerable shrinkage and deformation. According to Steinbrinck (5), the loss of water from a plant tissue not only decreases the size of the cells of the tissue and deforms them, but also affects the concentration of the cell sap which, during the giving off of water, pulls the cell membrane along with it. As the turgor becomes diminished, the membrane weakens and the cohesion of the concentrated cell sap draws the cell membrane along with it to the center, producing thus a decrease in volume of the tissue. This explanation seems applicable also to the decrease in volume and to the deformation of the cells of the leaf of the polypody in the curled state.

Concerning the physics of the curling and expanding of the leaf of this epiphytic polypody, the writer believes that the curling is not entirely an

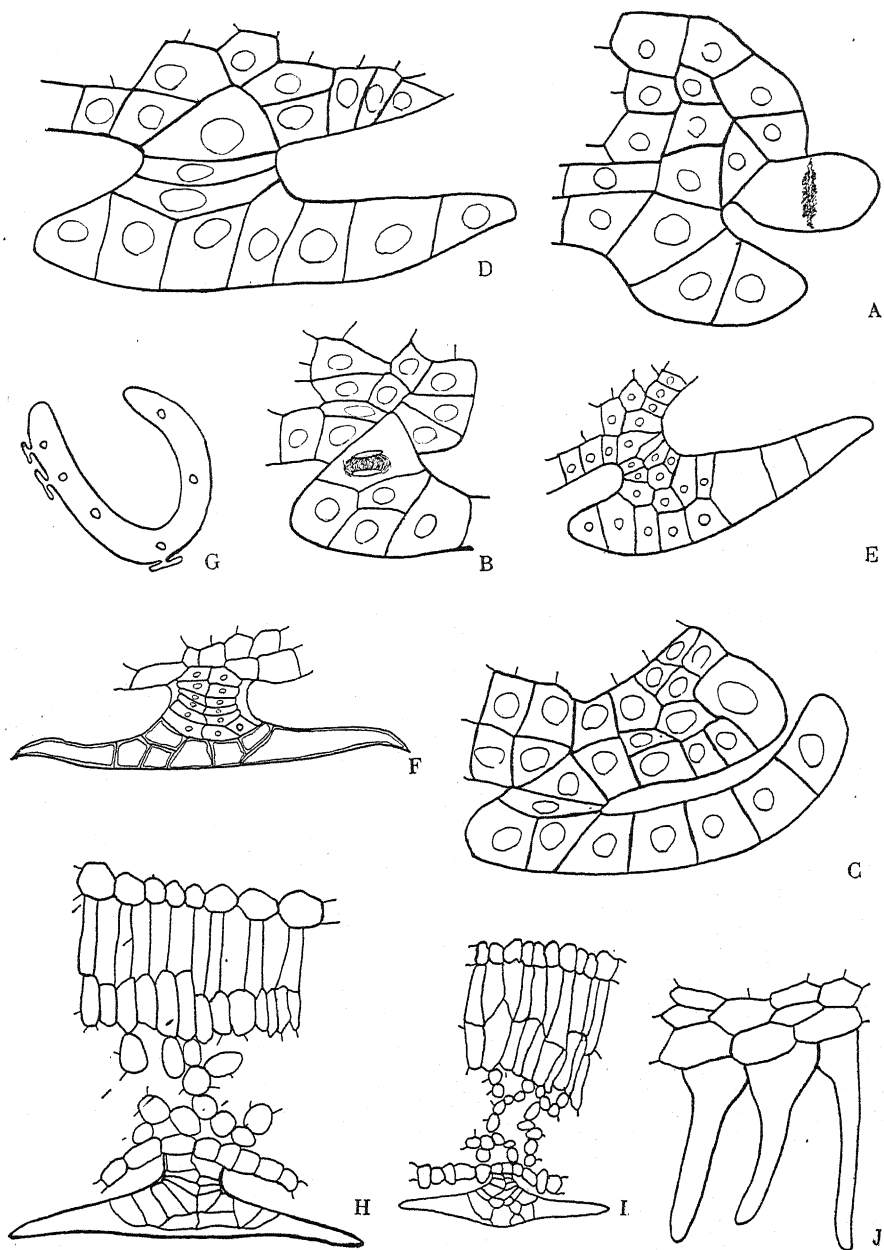


osmotic phenomenon. The loss of water from the cell walls themselves probably causes a shrinkage and a deformation of the cell wall. The so-called "resurrection" of the leaves is probably largely due to imbibition of water by the cell walls. This seems to be true from the behavior of these walls as it was observed under the microscope, in cross sections of fixed and imbedded leaves where no osmotic action could occur. These cells, many of them cut open so that turgor is impossible, expanded quickly to their normal plump form upon the introduction of water. In the living leaf, however, there is reason to believe that both osmosis and imbibition aid in the expansion of the leaf immediately after a rain. Undoubtedly, the process of expansion begins with an imbibition of water by the cell walls, and later the osmotic action carries the water from cell to cell within the leaf, thus aiding in the distribution of the water imbibed by the cell walls.

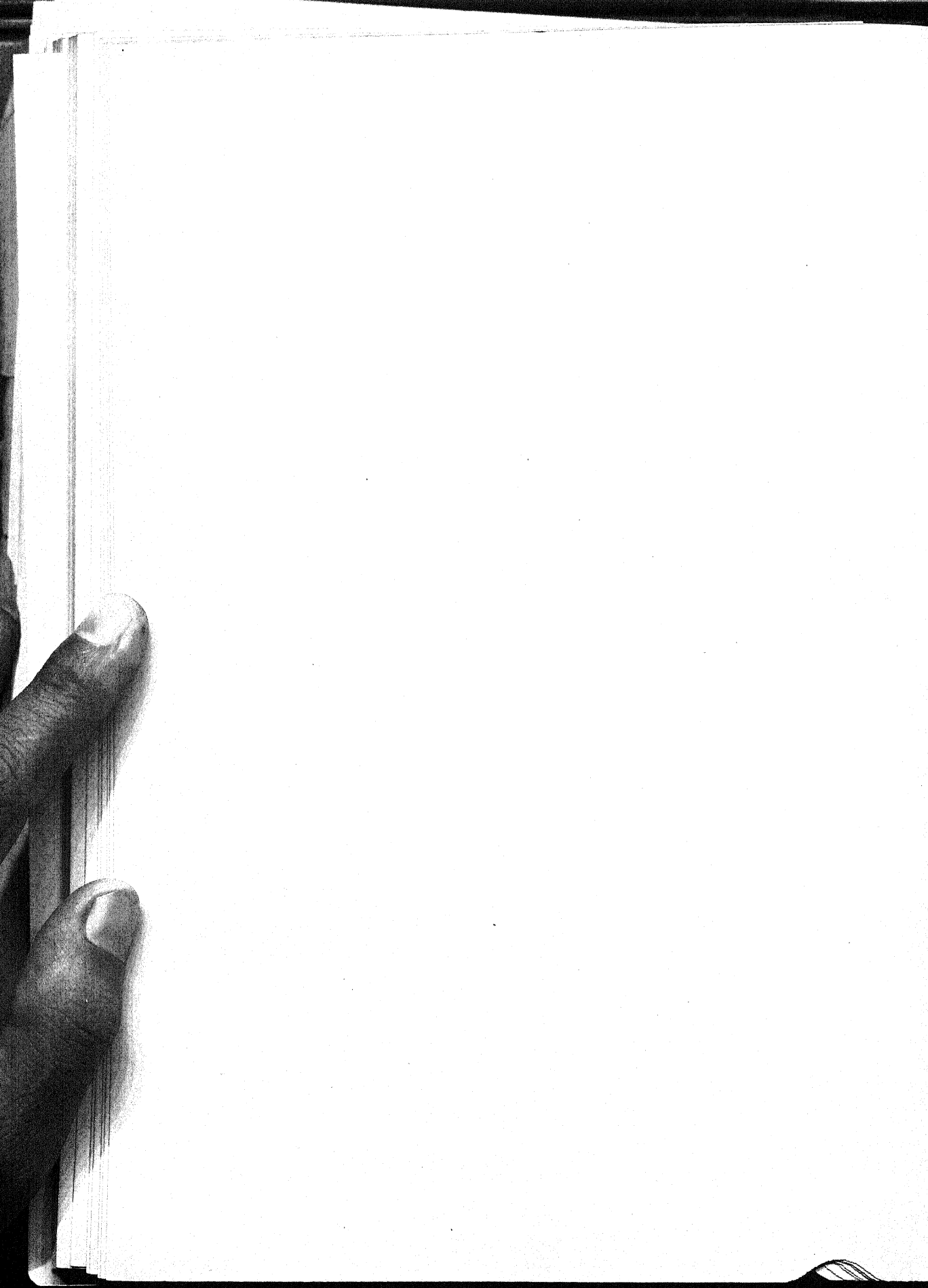
#### ANATOMICAL STUDIES

It will be well to describe the development and the mature structure of the scales of the leaf of the epiphytic polypody, since this knowledge should aid in solving the problem of their function. A sagittal section of a very young leaf of a mature plant, or of a young sporophyte, reveals the fact that each scale arises from an epidermal cell. The scale is, of course, initiated very close to the growing point of the leaf. The epidermal cell from which the scale arises first divides by a transverse wall, forming an inner and an outer cell. The inner cell remains on the level with the epidermal cells, while the outer one bulges out and soon divides by a second transverse wall, forming again an inner and an outer cell. The outer of these two cells continues to divide several times, forming a plate of cells, while the lower cell, by several successive transverse and one median longitudinal divisions, gives rise to the stalk of the scale. The cells of the plate grow out more rapidly towards the growing point of the leaf, so that in the mature scale the plate or wing of the scale is wider towards the tip of the pinna. The mature scale consists of a sunken funnel-shaped body, the lower end of which is sunken in the tissue of the leaf and its upper plate-like flat portion rests on the surface of the epidermis. The plate-like portion consists of a peripheral membranous, radially ribbed, parenchymatous wing and a central cluster of cells. The cells of the wing are thick-walled and devoid of protoplasm, as are also the cells of the central cluster except that the latter are brown in color. The cells of the funnel-shaped stalk of the scale, unlike those of the plate, are thin-walled and contain protoplasm, as is indicated by the presence of large nuclei. Several stages in the development of the scales are shown in Plate XXI, A-F.

A study of the root and of the root hairs of *Polypodium polypodioides* shows first of all no signs of mycorrhizal associations. The structure of the root as a whole seems to present the appearance of a typical fern root, though the root hairs differ somewhat from the ordinary type. Instead of being



PESSIN: LEAVES OF POLYPODIUM



more or less uniform in thickness, as root hairs generally are, these are swollen or bulbous at the base and taper off to a more or less blunt point at the tip, as shown in Plate XXI, *J*.

#### SUMMARY

1. The leaf of *Polypodium polypodioides* under dry conditions loses the greater amount of water from its lower surface, with the result that a curling of the leaf occurs, which leaves the lower epidermis exposed and the upper epidermis concealed on the inside.

2. The curling of the living leaf is apparently the result of osmotic phenomena; the expansion is, in the case of dead leaves, entirely due to imbibition, while in the living ones it is due to both imbibition and osmosis.

3. The function of the scales is probably to facilitate an equal and gradual distribution of water over the surface of the leaf after a rain, also to absorb water that is present on the surface of the leaf, and to pass it to the internal tissues of the leaf.

4. The scales of the leaf originate from epidermal cells near the growing point of each pinna.

5. No mycorrhizal fungus was seen in the roots of this fern.

The writer is indebted to Professors Burton E. Livingston and Duncan S. Johnson for valuable advice.

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#### EXPLANATION OF PLATE XXI

FIGS. *A*, *B*, *C*, *D*, *E*, and *F* show successive stages in the development of the scales ( $\times 1440$ ). FIG. *F* is a mature scale of an expanded leaf ( $\times 1050$ ).

FIG. *G*. Cross section of a curled leaf.

FIG. *H*. Cross section of expanded leaf ( $\times 850$ ).

FIG. *I*. Cross section of curled leaf ( $\times 850$ ).

FIG. *J*. Typical root hairs of *Polypodium polypodioides*.

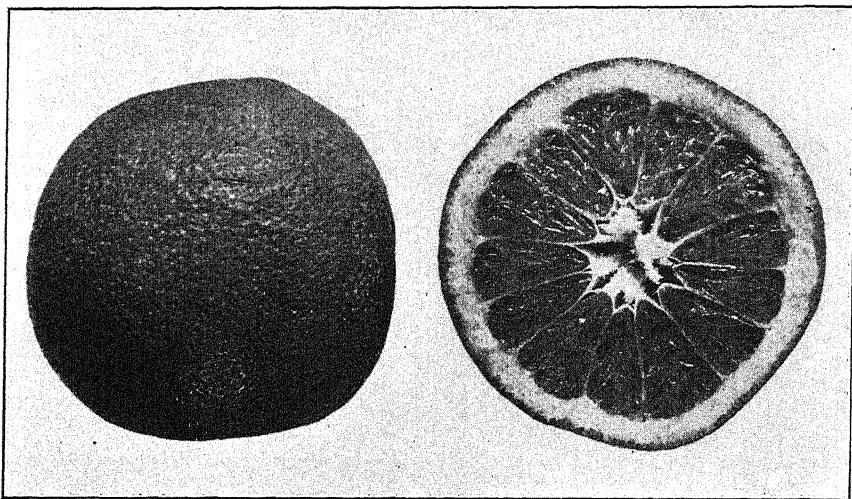
A CONSIDERATION OF THE SPECIES *CITRUS MAXIMA*  
(BURM.) MERRILL

ELMER D. MERRILL AND H. ATHERTON LEE

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The senior writer has previously shown that Burman in his "Index Universalis Herbarium Amboinense" used the binomial *Aurantium maxima* for Rumphius' description of the East Indian pummelo or shaddock. Burman definitely proposed this binomial in 1755, and the specific name is valid under all codes of botanical nomenclature. The description of this form by Rumphius, to which Burman referred his binomial, is in both Latin and Dutch and is very complete; the description is accompanied by a large plate showing both fruit and foliage, which leaves no room for doubt as to the identity of the plant under discussion. Burman's specific name for this form thus antedates all others and, following the conventions of the Vienna conference, has been accepted.

This species covers at least two distinct forms: the East Indian form known as the pummelo, shaddock, or, as the Javanese call it, the pomplum;

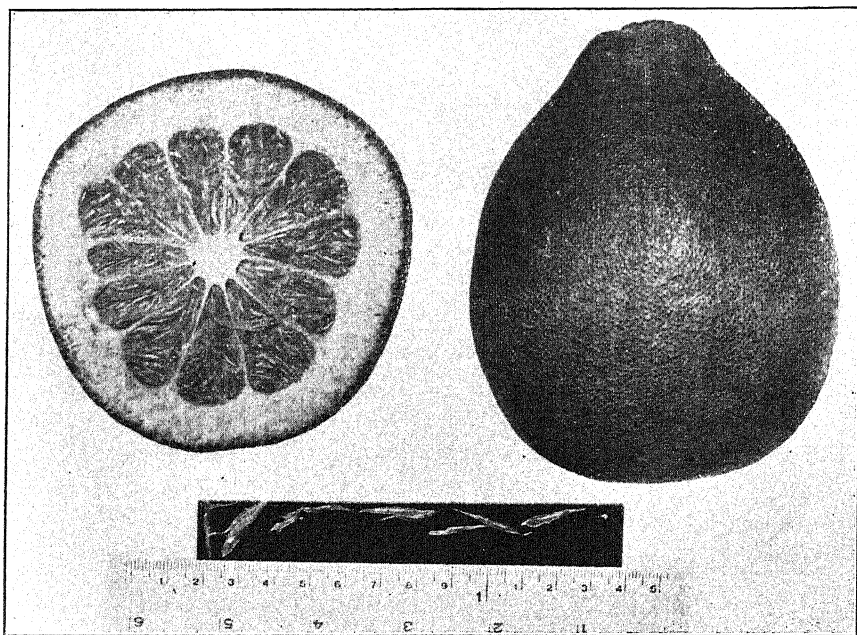


TEXT FIG. 1. The West Indian type of *Citrus maxima* (Burm.) Merrill, here described as *C. maxima* var. *wacarpa*, showing the small size, globose shape, small glands, thin skin, and small pulp vesicles as compared with the East Indian or pummelo type.

and the grapefruit, the latter being the form found in commerce in America, and grown largely in Florida, California, and the West Indies. These forms

exhibit very distinct differences in both fruits and foliage. In discussions involving the comparison of the East Indian forms of pummelo with the West Indian and American-grown grapefruit, it has been found very awkward to make the distinctions clear with the present nomenclature. Since the differences in these forms are distinctly well marked, and since the purpose of nomenclature is to define plant forms properly as an aid in botanical work, the writers suggest the formation of a botanical variety *Citrus maxima* var. *wacarpa* to include such forms as are known as the grapefruit in America and the West Indies. The description is as follows:

*Citrus maxima* var. *wacarpa* var. nov. GRAPEFRUIT. A medium-sized tree of spreading habit, spines absent, or if present small and slender;



TEXT FIG. 2. The East Indian type of pummelo or shaddock, pear-shaped, with large glands, thick skin, and large, coarse pulp vesicles. Photograph by the Philippine Bureau of Agriculture.

leaves broad, ovate; petiole with broad wings but not as broad as in the East Indian form; texture of the leaf also looser, more flexible, and thinner than in the case of the East Indian form, usually glabrous whereas the pummelo is frequently pubescent. Flowers axillary, either single or in clusters, usually smaller than in the East Indian form, with fewer oil glands on the corolla and such glands of smaller size. Fruits slightly oblate, in some cases, to globose and even slightly pear-shaped in others. Of smoother skin than the East Indian form, with smaller although more abundant glands. Usually lemon-colored although frequently continuing green in the tropics; skin but moderately thick, seldom more than 1 centimeter in

thickness, white and of a pithy, spongy substance. Flesh of smaller and more delicate vesicles than in the pummelo, of pale green color; walls of segments usually thin. Seeds abundant, moderate-sized. A photograph of this fruit is shown in text figure 1.

Grown commercially in Florida, California, Arizona, and the West Indies as a breakfast fruit. The horticultural varieties of the grapefruit are for the most part very susceptible to a serious disease of citrus plants known as citrus canker; in this respect they differ markedly from the East Indian pummelos, the latter being but moderately susceptible to citrus canker and in some cases approaching resistance.

It is furthermore suggested that the name employed in commerce for this botanical variety be continued as the *grapefruit*, discarding the attempt to use the word *pomelo* which seems artificial and confusing. The word *pummelo* or *shaddock* should then be employed to designate the thick-skinned, original East Indian type of this species only. These words are already largely in use in the East Indies to express this distinction. A photograph showing this East Indian type appears in text figure 2.

PHILIPPINE BUREAU OF SCIENCE



# THE HELMINTHOSPORIUM DISEASE OF RICE OCCURRING IN THE SOUTHERN UNITED STATES AND IN THE PHILIPPINES

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## INTRODUCTION

The Helminthosporium disease of rice<sup>2</sup> (*Oryza sativa* L.) is primarily a seed-bed trouble arising from the planting of infected grains. The disease may occur on any part of the rice plant at any time during the entire life of the plant. In May, 1918, the writer first collected diseased rice seedlings in Los Baños, Philippine Islands. In the fall of 1920, Dr. W. H. Tisdale, then in charge of rice-disease investigations, Bureau of Plant Industry, United States Department of Agriculture, collected infected rice panicles from the Crowley Rice Station in Louisiana.

Outside of Java and Japan, the Helminthosporium disease of rice occurs in Italy (4), in the Straits and Federated Malay States (5), in the Dutch East Indies (15), in the Philippines (13), in southern China (14), in Louisiana, in India (16), and Dr. R. D. Rands collected diseased specimens from Sumatra.

## THE DISEASE

### Economic Importance

In Japan, Nishikado and Miyake (7, 8) state that in seed-beds the disease may attack about 90 percent of the seedlings, and that sometimes practically all of the plants are infected. In germination experiments these authors report 12.5 percent of the seedlings affected by the disease (9).

In Italy, Farneti (4) states that *Helminthosporium oryzae* and *Piricularia oryzae* are different names for the same species, for which the generic name

<sup>1</sup> The author acknowledges his indebtedness to Professor L. R. Jones of the Department of Plant Pathology of the University of Wisconsin, under advisory relations with whom this work was done. Thanks are also due to Doctors A. G. Johnson and W. H. Tisdale of the Bureau of Plant Industry, United States Department of Agriculture, for counsel in connection with the investigations, and to Dr. W. T. Swingle of the same bureau for abstracts of the Japanese literature. To Doctors J. G. Dickson and E. M. Gilbert of the University of Wisconsin he is indebted for much assistance respectively on the physiological and the mycological aspects of the problem.

<sup>2</sup> In an abstract (Phytopath. 12: 34. 1922) the writer called this disease the "sesame spot disease of rice." The name is here changed to "Helminthosporium disease of rice," which includes all forms of the malady, to make it distinctly a disease of rice and to avoid possible confusion with the Helminthosporium disease of *Sesamum indicum* L. caused by *Helminthosporium sesameum* Sacc., which is widely distributed in the Philippines.

Piricularia is preferred. *P. oryzae* is regarded as the most destructive of the rice fungi.

In the Philippines, during the growing seasons of 1918 and of 1919, the writer found that from 10 to 58 percent of the plants were killed by *Helminthosporium oryzae* (10). The remaining living plants were stunted and weakened, and finally they were attacked by other fungi, commonly *Coniosporium oryzinum* Sacc. and a species of *Acrothecium*, to which they finally succumbed.

In Crowley, Kaplan, and Chueydan, Louisiana, the writer observed that the disease was present on practically all cultivated rice in the field in September, 1922, although the occurrence of the disease does not attract much attention.

In the Philippines and in Louisiana, the writer noted the different degrees of infection of the various varieties of rice planted side by side. From these observations it seems probable that some varieties of rice are more resistant to the disease than others. According to Nishikado and Miyake (9), similar observations were reported by Suematsu in Japan in 1921.

Sundararaman (16) states that the *Helminthosporium* disease of rice produced heavy damage in India in 1918 and 1919 on account of the heavy rains at heading time.

#### Symptoms

*Early stage of infection.* The first symptom of the disease in the seedling stage is the presence of abundant circular and oval spots on any part of the seedlings above ground (Pl. XXII, A). When the lesions are young, they are light brown with a rather water-soaked margin and are very distinct on the leaves. As the spots enlarge and become old, the centers turn gray with distinct dark-brown borders. The circular patches vary in size from 0.2 millimeter to 2.0 millimeters across, and the elongated ones from 0.5 millimeter to 3.0 millimeters long by 0.5 millimeter to 1.0 millimeter wide. Infected rice collected from Louisiana and germinated at Madison, Wisconsin, produced the same lesions on the seedlings. On the shoots of the seedlings the lesions grow very rapidly, forming a band around the stem and killing the entire upper portion of the plant.

*Advanced stage of infection.* In older stages of infection two or more spots may join together and become confluent dead patches similar to those described in Japan (17). The lesions are fairly distinct even after the death of the infected parts (Pl. XXIV, A, 1, 2, 3; B, 1, 2). The belts formed around the stems of the seedlings rot, and all parts above them turn yellow and die. Following the death of the seedlings, a dull black mass of fruiting bodies is formed all over the dead portion.

Specimens<sup>3</sup> of infected panicles collected by Dr. Tisdale in 1920 and

<sup>3</sup> Thanks are due to Dr. W. H. Tisdale for the first materials of the Louisiana strain of *H. oryzae*, to Mr. Y. Nishikado of the Ohara Institute for Agricultural Research, Kuraschiki, Okayama, Japan, for specimens of the Japanese strain of *H. oryzae*, to Dr. A. G. Johnson

by the writer in 1922 showed a black mass of conidia and conidiophores on the grains, in some cases completely enclosing the caryopses. Infected panicles collected by Dr. Rands from Java and Sumatra showed symptoms identical with those of the specimens collected from Louisiana (Pl. XXII, C, 1, 3, 5). Infection of culms and glumes has also been reported in Japan (9, 17).

In India, Sundararaman (16) describes the symptoms of the *Helminthosporium* disease of rice occurring there as small spots 1.2 to 14 millimeters by 0.5 to 3.0 millimeters. The centers turn smoky black in consequence of the production of sporophores.

#### THE CAUSAL ORGANISM

The fungus was isolated by Keitt's single-spore method of isolation (6). All stock cultures, as well as those used for artificial inoculation work on aerial parts of seedlings and of mature plants, were grown on potato-dextrose agar.

#### Morphology

*Mycelium.* The mycelia of the Philippine and of the Louisiana strains of *Helminthosporium oryzae* are light brown, fine, fairly uniform in width, branched, septate, varying in width from 3 to 8  $\mu$ . The length of the cells varies from 140 to 440  $\mu$ .

*Conidiophores.* The conidiophores of the Philippine and the Louisiana strains of *H. oryzae* are aerial in fascicles of two to four, dark brown, lighter-colored towards the apex, slightly constricted at the base, with the basal cell swollen, more or less flexuous, 7- to 26-septate, and 200-600 x 4-8  $\mu$  (Pl. XXVI, figs. 1, 2).

*Conidia.* The conidia of both strains are borne apically or on knee-like projections on the sides of the conidiophores. They are light brown or olivaceous, slightly lunate, fusoid, fusoid-cylindrical, obclavate, or clavate, 5- to 11-septate, not constricted at the septa, with contents finely granular, obtuse at both ends, readily detached, 45-120 x 10-20  $\mu$ , germinating at both extremities (Pl. XXVI, figs. 1, 2), occasionally germinating in the middle cells (Pl. XXVII, figs. 3, 6).

Tanaka (17) gives Hori's original and revised descriptions of the Japanese *H. oryzae*. Nishikado and Miyake (9) give Breda de Haan's description of the strain in Java.

A count made from a total of 200 conidia from pure cultures and 199 conidia from field material of the Louisiana strain showed that in pure culture the largest class of the conidia have nine cells. In the field material, the largest class have seven cells. The Philippine strain of *H. oryzae* has from six to ten cells in the conidia.

for materials of the Hindu strain of *H. oryzae* which was determined by Mr. M. Mitra of Pusa, India, and to Dr. R. D. Rands for specimens collected in Java and Sumatra.

*Germination of conidia.* Germination of conidia, using field material of the Philippine and the Louisiana *H. oryzae*, showed that a germ tube appears from one end or one from each pore in from 30 minutes to 10 hours, depending upon the age of the specimen. After 26 months, germination of the conidia took place after 24 hours in distilled water at room temperature. Nishikado and Miyake (9) report the minimum temperature for the germination of conidia of the Japanese strain of *H. oryzae* as 2° C., the optimum as near 30° C., and the maximum as 41° C. In culture these authors report that the fungus remains alive for two years and seven months.

It was noted that, of 309 conidia mounted in sterile distilled water in Van Tieghem cells, 193, or 62.46 percent, germinated by the production of a germ tube from each of the two ends, and 101, or 32.69 percent, produced a germ tube from one pole only at room temperatures. Fifteen of the conidia were broken. These broken conidia produced a germ tube from the broken end, the sound end, or from both. Sundararaman (16) states that a germ tube may sometimes appear from the middle one or two cells of the conidia of the Hindu strain. Nishikado and Miyake (9) state that an increase in the proportion of germination from the middle cells of the conidia follows a rise of temperature.

*The production of conidia in culture.* Conidia of both the Louisiana strain AIS<sup>4</sup> and the Japanese strain JNS1<sup>5</sup> were mounted on potato-dextrose agar. The medium was diluted with twice its volume of distilled water. By means of the platinum loop, drops of the melted agar were placed on sterile cover slips. The drops were inoculated with conidia by means of the tip of a sterile platinum needle. After inoculation, the cover slips were inverted over Van Tieghem cells to which they were fastened with vaseline, leaving open spaces for aëration. At room temperatures, both strains germinated after 30 minutes. The Japanese culture produced conidia in 24 hours, and the Louisiana strain had well formed conidia after 48 hours. At the end of each branch of the hypha a conidium was produced. The conidia have the characteristic shape, color, and granular contents

<sup>4</sup> Sporulating cultures of the Louisiana strain of *H. oryzae* are described under "Physiology."

<sup>5</sup> The writer wishes to thank Dr. F. L. Stevens of the University of Illinois and Mr. Y. Nishikado of Kuraschiki, Okayama, Japan, for cultures of the Japanese strain of *H. oryzae*.

(1) Cultures received from Dr. Stevens:

JNS1.....	Culture	45 of Mr. Nishikado
JNS5.....	"	142
JNS7.....	"	144
JNS8.....	"	139

(2) Cultures received from Mr. Nishikado (see Nishikado and Miyake (9) for their history):

JN1.....	Culture	45, tube 1
JN2.....	"	45, " 2
JN3.....	"	63
JN4.....	"	83
JN5.....	"	142
JN6.....	"	143
JN8.....	"	139

of those produced in the field, except that they are smaller (Pl. XXVII, figs. 5, 8). At the end of 70 hours, from two to four conidia were borne at the apex of each conidiophore-like hypha. The portions of the hyphae functioning as conidiophores began to turn brown or olivaceous at the end of 70 hours. After 120 hours, the color of the conidiophores was very distinct brown and the granulations in the cells of the conidia could be easily distinguished. Fusion of cells of adjacent hyphae, as in other higher fungi, was also noted (Pl. XXVII, figs. 2, 9).

To place as much of the fungous growth in the same plane as possible, ordinary potato-dextrose agar was smeared as thinly as could be on clean and flamed slides. The agar smears were inoculated with a loopful of a suspension of the conidia of the Louisiana strain AIS and the Japanese strain JNSr of *H. oryzae* in sterile distilled water. The slides were incubated in moist chambers at room temperature. Miss Bachman (1) in 1918 described a method of staining yeast cells in cultures on slides. She used micro-formol and Flemming's weak solution diluted one half to fix the fungus, and stained the slides after fixation. The writer fixed the cultures on the agar smears with Flemming's medium solution diluted one-half. The slides were fixed in Koplins jars for 24 hours, after which they were washed for 48 hours and run through the alcohols up to 95 percent without removing them from the jars. Then the slides were bleached over night, washed, and stained with Heidenhain's iron-alum haematoxylin and Flemming's triple stain. From the stains the slides were transferred to absolute alcohol, cleared with clove oil, and mounted in Canada balsam. The results were satisfactory for the study of the method of conidium-formation in culture. Heidenhain's iron-alum haematoxylin is preferable to the triple stain for the reason that the agar does not stain with the former.

In the experiments it was noted that the Japanese strain produced conidia in 24 hours and the Louisiana strain in 48 hours. All other successive stages are identical, except that the Louisiana strain requires a longer time than the Japanese for its development.

### Taxonomy

The *Helminthosporium oryzae* occurring in Java was described by Breda de Haan (2) as having fusiform conidia. Hori described the Japanese strain as having obclavate conidia (9, 17), and Hara, also in Japan, described obclavate or fusiform conidia (9). The Philippine fungus has fusoid to obclavate spores, and the Louisiana strain has fusoid, fusoid-cylindrical, or obclavate to clavate conidia. It was observed from different mounts of the spores of both the Philippine and the Louisiana strains that under field conditions conidia of various shapes and sizes are produced. It is probable that the different environmental conditions under which the various strains are growing exert an important influence upon the form and size of the conidia produced, as shown by the seemingly transitory shapes.

The writer believes that the size of the conidia is of minor importance. Many factors, such as local meteorological conditions, age of the conidia when the specimens were collected, weather conditions at the time of their production, and the number of spores measured, have a great deal to do with the size of the conidia reported by different observers. The writer's figures for the conidia and conidiophores of the Javanese, based on 50 conidia and 20 conidiophores, approach those of the Philippine and the Louisiana strains.

In the present study the writer concludes that the Louisiana and the Philippine strains are identical with each other and with the Japanese fungus. Since the Japanese fungus is identical with that described by Breda de Haan in Java, the four strains belong to the same species—*H. oryzae* Breda de Haan.<sup>6</sup>

### Physiology

The writer found that the Philippine and the Louisiana strains of *Helminthosporium oryzae* sporulate sparingly on potato-dextrose agar, oatmeal agar, cornmeal, oatmeal, ground rice, ground beans, rice-glucose agar, and rice-leaf-decoction agar.

*Production of conidia on plant tissues and on artificial-media.* Beginning in November, 1920, experiments were conducted to determine the relation of various plant tissues to the production of conidia by both the Philippine and the Louisiana strains of *H. oryzae*. The plant tissues used were immature tomato stems, canned asparagus tips, canned string beans, dry corn stalks, young and immature corn leaves and stems, empty and fertile rice panicles, green and dry stems of *Sambucus canadensis* L., fertile sorghum panicles, dry soybean pods, dried stems of *Melilotus alba* Desr., immature bean pods, dry piths of old corn, dry rice culms, dry leaves of *Poa pratensis* L., pine blocks, turnip plugs, immature apple-fruit plugs, radish plugs, beet plugs, celery petioles, kohlrabi plugs, and sweet corn ears. All were cut into pieces about five centimeters long, and tubed with cotton at the bottom. The tubes were sterilized in the autoclave for 30 minutes at 10 to 15 pounds' pressure.

In the experiments it was found that both the Philippine and the Louisiana strains of *H. oryzae* produced conidia on sterilized tomato stems, rice and sorghum panicles, dry rice culms, apple-fruit plugs, turnip plugs, and radish plugs. The Louisiana strain produced conidia on young and immature corn leaves and stems, empty rice panicles, stems of *Sambucus canadensis*, stems of *Melilotus alba*, and dry corn piths. Only the Philippine strain produced conidia on celery petioles. The Louisiana strain produced free-fruiting cultures AUR and AIS on rice and sorghum panicles respectively.

<sup>6</sup> In an abstract (Phytopath. 12: 34. 1922), the writer has called attention to the priority of Breda de Haan's description of *Helminthosporium oryzae* over Miyabe and Hori's description.



The Louisiana strain also produced conidia on cotton moistened with a decoction of 100 grams of either hulled or unhulled rice in 500 cc. of water. The decoction was prepared by steaming the rice after the addition of the water in an Arnold steamer for an hour. The liquid was decanted and filtered through cotton while hot. The absorbent cotton in test tubes was moistened with the decoction, and the tubes were plugged and autoclaved 30 minutes at 15 pounds' pressure.

Two other solutions were used for moistening filter paper on which *H. oryzae* was grown. These solutions were Pasteur's nutrient solution and sheep-manure decoction. Pasteur's nutrient solution, modified, was prepared as follows:

Distilled water.....	100 cc.
Potassium acid phosphate, $\text{KH}_2\text{PO}_4$ .....	0.5 g.
Tribasic calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$ .....	0.05 g.
Magnesium sulfate (crystalized), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.5 g.
Ammonium tartrate, $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$ .....	1.0 g.

The solution was divided between two 150-cc. Erlenmeyer flasks and heated in the steamer for 20 minutes on three successive days. Folded filter paper, in test tubes which were autoclaved 30 minutes at 15 pounds' pressure, was moistened with the solution and allowed to stand 48 hours in order to eliminate any contaminations that might develop. The filter paper was inoculated with the mycelium of the Louisiana and the Philippine *H. oryzae*. Three months later it was found that conidia were produced on the filter paper.

The other solution was sheep-manure decoction prepared as follows: Filter paper, sterilized as in the preceding experiment, was moistened with a decoction of sheep manure prepared by stirring 20 grams of well rotted sheep manure in 1000 cc. of water for 20 minutes. Four grams of dextrose were added, and the liquid was filtered through filter paper. After the filter paper in the test tubes was moistened, the test tubes were autoclaved 30 minutes at 15 pounds' pressure. The tubes were inoculated with mycelium of *H. oryzae*, and conidia were produced on the filter paper.

The conidia on the plant tissues, on the cotton moistened with the plant-tissue decoction, and on the filter paper moistened with the Pasteur's solution and with the manure decoction, invariably yielded non-sporulating cultures when transferred to new potato-dextrose agar. In 1921, two sporulating strains were obtained from transfers made from the conidia on the cotton at the bottom of test tubes containing rice and sorghum spikelets. These gave rise to strains AUR and AIS respectively. The third sporulating strain of the Louisiana material was produced from spore strain "O" grown on potato-dextrose agar. The conidia produced by this culture gave rise to the strain AO.

As plant tissues did not give uniform results in the production of conidia of *H. oryzae*, the effect of a few carbohydrates added to water agar was



tested. Five grams each of inulin, potato starch, sucrose, maltose, and lactose were placed in separate one-liter flasks, and to each flask five grams of agar flour and 500 cc. of distilled water were added. The mixture was thoroughly agitated, and the flasks were plugged and autoclaved for 25 minutes at 10 to 15 pounds' pressure. The flasks were again vigorously agitated to insure a thorough mixture, and the media were tubed without filtration. The tubes were plugged and autoclaved for the same length of time and at the same pressure. The tubes were inoculated with sporulating and non-sporulating strains of the Philippine, Louisiana, and Japanese strains of *H. oryzae*. After 35 days it was noted that the sporulating cultures AIS of the Louisiana strain and JNS1 of the Japanese *H. oryzae* produced abundant conidia on all the carbohydrate agars. Both these strains grew sparingly on inulin agar. The non-sporulating Louisiana strain AN produced only very few conidia on all the agars. The Japanese culture JNS5 produced conidia sparingly on inulin. Culture JNS7 produced conidia on sucrose agar but not on the others. The Japanese culture JNS8 produced conidia on lactose, inulin, and sucrose agars, but not on the maltose and potato-starch agars. The Philippine culture sporulated on all except sucrose agar.

Another experiment was conducted to find a medium on which *H. oryzae* will produce abundant conidia. In this test, rice agar, to which a few of the rare carbohydrates had been added, was used. The substances used were chemically pure d-mannose, l-arabinose, levulose, erythritol, galactose, xylose, commercial dextrin, and hydrated rhamnose. The different rice agars were prepared according to the following directions, substituting each of the substances for the d-mannose.

**Solution A.** In a two-liter Erlenmeyer flask, 100 grams of unhulled rice were covered with 1000 cc. of tap water. The flask was autoclaved for one hour at 15 pounds' pressure. After cooking, the liquid was filtered through cotton while hot, and the filtrate was brought up to the original volume of 1000 cc. by the addition of tap water:

**Solution B.** In a liter Erlenmeyer flask, 15 grams of Bacto agar were covered with 750 cc. tap water. The flask was steamed in the Arnold steamer for one hour, being shaken from time to time to insure a thorough mixing. When the agar was melted, it was filtered while hot through cotton, and the volume was made up to 750 cc. with tap water. To solution B, 250 cc. of solution A and 20 grams of d-mannose were added, and the mixture was steamed for 10 minutes with shaking. The medium was tubed, plugged, and autoclaved 35 minutes at 10 to 15 pounds' pressure.

Transfers were made into tubes of each of the rice agars from mycelium and conidia of 26-day-old potato-dextrose-agar cultures of the Louisiana strains AIS and AI, of the Japanese cultures JN1 and JN5, and of the Philippine culture PO5 of *H. oryzae*. At the end of 16 days it was noted that only the sporulating strains JN1 of the Japanese and AIS of the

Louisiana *H. oryzae* produced conidia on all the media. On water agar, which was used for a check, the growth was hardly visible. On this, and on rice agar to which nothing was added, only the sporulating cultures AIS and JN1 produced conidia.

The effect of low concentrations of tri-hydroxy alcohol on the behavior of *H. oryzae* was also tested. For this experiment, three-, four-, and five-percent glycerin agars were prepared by adding to each of three 250-cc. portions of 1.5-percent water agar, 7.5 cc., 10 cc., and 12.5 cc. respectively of chemically pure glycerin. The flasks were autoclaved for 30 minutes at 12 pounds' pressure and thoroughly shaken. After autoclaving, the agars were tubed, plugged, and sterilized at the same pressure for the same length of time and the tubes were slanted. Transfers were made into two tubes of each from mycelium of non-sporulating cultures and from conidia of 10-day-old free-fruiting cultures on potato-dextrose agar, representing the Philippine, Louisiana, and Japanese *H. oryzae*. The tubes were examined when the transfers were 66 days old. Only the sporulating cultures JNS1 of the Japanese, and AIS, AUR, and AO of the Louisiana strain produced conidia. The Philippine non-sporulating culture PO produced abundant conidia on four-percent glycerin agar and fewer on three and five percent. In regard to the production of spores the Japanese and the Louisiana strains are more alike in their behavior on glycerin agar. The Philippine and the Japanese strains of *H. oryzae* color the agar in the same degree, while in the Louisiana strain only a trace of coloration was observed.

*The effect of light, temperature, partial desiccation, and irritation on the production of conidia.* The amount of conidia produced on sterile plant tissues was very limited and the method required a long time. A means which would give a quicker result was resorted to. This was especially necessary before sporulating cultures were developed. Rands' method (12) with a slight modification was followed for inducing the formation of conidia. The writer used Soxhlet flasks, each provided with an inverted U-tube, in place of petri dishes. One of the arms of the U-tube was inserted into the flask through the cotton plug, and the other arm was used as an exit for the water vapor when the flasks were exposed to the sun. The U-tubes, of about seven-millimeter glass tubing, were inserted after the flasks were filled one third full of 1.5-percent potato-dextrose agar. The outer ends of the U-tubes were plugged with cotton, and the flasks were sterilized for 30 minutes at 15 pounds' pressure. When the agar congealed, a small piece of mycelium was transferred into each, and the fungus was allowed to grow for 12 days at room temperature. At the end of the incubation period, the culture was cut into small pieces by means of a sharp, sterilized scalpel. The pieces were thoroughly mixed, the plugs were removed, and the flasks were exposed to the sun or to a 220-volt electric lamp.

After a few minutes of exposure, water vapor began to condense on the

inner walls of the flasks. Part of the vapor passed through the exit tubes, and the rest collected at the bottoms of the flasks. On the fourth day after exposure of about nine and a half hours to the sun the cultures had abundant conidia. In artificial light it took two to three days of continued exposure for the conidia to develop on the pieces of agar containing fungous mycelium. The flasks kept inside the laboratory at room temperature did not produce conidia.

Exposing the cultures to the sun in early spring, when the maximum temperature rarely exceeds 20° C., or in midsummer, when the temperature within the flask is above 40° C., did not induce conidium-formation. Although no extensive work has been carried out to determine the relation of temperature to the formation of conidia, the results seem to indicate that a temperature a little above the optimum for the development of the fungus in artificial culture is essential for conidium-production. According to Nishikado and Miyake (9), the fungus produces conidia readily at 35° C. but not at from 39° C. to 40° C.

*The relation of temperature to the growth of Helminthosporium oryzae.* Cultures on potato-dextrose agar in petri dishes were incubated in the temperature tanks with temperatures at four-degree intervals from 20° C. to 40° C. In the first experiment, only the Louisiana strain AIS was used. After 70 hours it was found that the cultures grew best at 28° C. reaching a diameter of 24 mm., at 24° C. reaching 20 mm., at 32° C. reaching 18 mm., at 20° C. reaching 12 mm., at 36° C. reaching 11 mm., and at 40° C. reaching 5 mm. (Pl. XXV). This experiment was repeated with the Louisiana culture AIS, the Philippine culture PO, and the Japanese culture JNSi, with identical results. In the second experiment, cultures were placed at 16° C.; at this temperature the growth was less than at 20° C. The results of these experiments are given in table I.

TABLE I. *Growth of Helminthosporium oryzae on Potato-dextrose Agar in Petri Dishes at Different Temperatures*

Strains Used	Age when Examined	Diameters of Growth (in millimeters)						
		16° C.	20° C.	24° C.	28° C.	32° C.	36° C.	40° C.
Louisiana, AIS.....	70 hrs.	.....	12	20	24	18	11	5
Louisiana, AIS.....	3 days	16	30	36	40	40	12	.....
Philippine, PO.....	3 days	16	24	40	40	40	12	.....
Japanese, JNSi.....	3 days	18	28	36	40	40	12	.....

Table I shows that *H. oryzae* grows best at temperatures from 24° C. to 32° C. The production of conidia by the sporulating strains takes place at from 16° C. to 28° C.; between 32° C. and 36° C. there is a tendency for the fungus in the culture to produce aërial mycelium. The result shows that the optimum temperature for growth of *H. oryzae* in artificial culture on potato-dextrose agar is around 28° C. Nishikado and Miyake (9) report the

optimum temperature for germination of conidia, and for the growth of the mycelium of the Japanese *H. oryzae*, to be 25° C. to 30° C. or close to 30° C.

*The relation of the hydrogen-ion concentration of the medium to the production of conidia by Helminthosporium oryzae.* Potato-dextrose agar adjusted to reactions of pH 1.2-1.4, 1.8-2.0, 2.4-2.6, 4.2-4.4, 7.3, and 8.6-8.8 was used in the experiment. The colorimetric method described by Clark and Lubs (3) was followed. The maximum alkalinity of the medium at which growth is possible was not determined. As the fungus did not produce conidia at any of the different reactions of the medium, no further work was done. The best vegetative development was obtained at a pH-concentration of 8.6-8.8, and only a bare growth of mycelium was obtained at a pH-concentration of 2.4-2.6.

#### LIFE HISTORY OF HELMINTHOSPORIUM ORYZAE IN RELATION TO THE PRODUCTION OF DISEASE

##### Mode of Overwintering

Infected grains of Philippine varieties of rice were examined microscopically, and it was noted that conidia frequently adhere to the surfaces of the lemma and palea of the grains which are apparently healthy, and that these spores are often caught between the trichomes. Hyphae are present running lengthwise in the grooves of the hulls (Pl. XXVII, fig. 6). One of the varieties of rice examined showed 555 out of 1000 grains, or 55.5 percent, with the brown mycelium on the hulls. This percentage does not include the grains which might have had the mycelium within the hulls.

In order to trace the mycelium within the caryopses, grains were immersed in a 10-percent sodium hydroxid solution to soften the hulls. After 21 hours, the endosperm was completely gelatinized and the hulls were sufficiently soft to work with. Microscopic examination of both outer and inner surfaces of the palea and lemma showed the presence of mycelium of *H. oryzae*. After the alkali treatment for 21 hours, the ovary wall readily separated as a solid membrane from the gelatinized endosperm. Microscopic examination of the ovary wall also showed the presence of the mycelium on the outer surface. On the hull as well as on the ovary wall of the rice grains the fungus produces star-shaped appressoria (Pl. XXVII, fig. 6).

The endosperm became gelatinized when the rice grains were soaked in the alkali solution for 16 to 21 hours. The starch grains in the cells were dispersed when the kernels were immersed from four to six hours in alkali solution. Hence it was not possible to distinguish fungous parts within the tissue enclosed by the ovary wall.

A series of isolations on sterile cornmeal, made by plating parts of the palea and lemma as well as hulled grains which had mycelium, showed that the fungous mycelium on and within the tissues of the grains is that of *H. oryzae*. The fungus is seed-borne as dormant mycelium (10), and is carried in the seed from season to season.

When infected grains are planted, a serious blight infection occurs before and after the seedlings emerge. As these infected seedlings die, the fungus produces abundant conidia. The conidia are blown over to rice in neighboring plots or fields, causing secondary infection (10).

#### The Effect of Exposure of Infected Rice Tissues upon the Appearance of other Spore Forms

Since the winter of 1920-21 experiments have been conducted to determine whether *Helminthosporium oryzae* on plant tissues could be induced to form spores other than those of the conidial stage. Infected panicles, rice seedlings, leaves, sheaths, and culms were exposed outdoors during the winter. These infected parts represented various stages of the disease from the time when the first symptoms appeared to the time when abundant conidia had been formed. Some of the materials were hung in wire baskets in order not to touch the ground. Others were placed on the ground, or left in the cans and pots in which they were growing. None of the infected tissues produced spores other than those of the imperfect stage. Exposure of infected panicles during the winter in Wisconsin destroyed the viability of the grains. Test tubes containing cultures on various plant tissues also failed to produce the perfect stage.

#### Spore-dissemination

In order to find if the conidia are carried in the air, causing secondary infection, especially during flowering time, petri dishes with sterile potato-dextrose agar were exposed for two to four minutes over rice flowers near an infected patch in the Philippines at about noon. It was noted that conidia of *H. oryzae* were among the spores caught. The fungus was induced to produce conidia, and inoculation experiments with the spores showed characteristic lesions on rice seedlings in 24 hours.

#### Pathogenicity

At Madison, Wisconsin, the comparative pathogenicity of the Philippine and the Louisiana strains of *H. oryzae* was tested on seedlings grown under sterile conditions, as well as on seedlings grown out of doors and in the greenhouse during the summer, and on different parts of the plant representing various stages of its development (Pl. XXII, A, B, C; Pl. XXIII, A, B; Pl. XXIV, A, B), beginning in 1921. In the inoculation experiments with seedlings grown under sterile conditions, symptoms became evident on the leaves and stems in from 20 to 24 hours. To grow rice under sterile conditions, the grains were hulled by pinching off the upper parts of the palea and lemma of the caryopsis, opposite the embryo, with the thumbnail of the left hand and pressing with the nail of the right hand in order to force out the kernel. The hulled grains were covered with a solution of 1:1000

mercuric chlorid for five to ten minutes, and were washed in three to five changes of sterile distilled water. After washing, the water was drained off and the kernels were left in the test tubes which were held in a horizontal position. After 16 to 24 hours in this position, the kernels were plated out on water agar under aseptic conditions. As soon as the seedling roots were two to four centimeters long, each seedling was transferred into a 14-inch test tube containing Knop's solution stiffened by the addition of 1.0 percent agar shreds and sterilized in the autoclave for 35 minutes at 10 pounds' pressure. In transferring the seedlings, a sterile platinum loop with a long handle was used for taking the seedlings by the shoots. To inoculate the seedlings in the test tubes, a suspension of conidia was applied on the leaves and stems by means of a platinum loop. The results show that the Philippine and the Louisiana strains of *Helminthosporium oryzae* are capable of producing the lesions on rice seedlings independently of other organisms.

In artificial inoculation experiments on aerial parts of rice, a conidial suspension in sterile distilled water was made from 15- to 30-day-old potato-dextrose-agar cultures. The spore suspension was applied to the rice with sterile atomizers. Checks, atomized with sterile water, were always run, and these remained healthy until the end of the experiments. The inoculated plants and the checks were covered for at least 24 hours to promote the germination of the conidia.

The summary of results on inoculation experiments conducted in Madison, Wisconsin, during the summer of 1921, using the sporulating strain AIS, are given in table 2.

In table 2 it is shown that the Louisiana strain of *H. oryzae* is as capable of infecting all parts of the rice above the ground at any stage of the entire life history of the plant as is the Philippine strain.

In the summer of 1922, when dry conidia from 15-day-old potato-dextrose-agar cultures of both the Louisiana strain AIS and the Japanese strain JNS<sub>1</sub> were rubbed on the upper surface of the leaves of mature plants by means of the platinum loop, they produced infection in 24 hours. In Japan, the incubation period is reported as 24 to 48 hours (9). Penetration of the germ tubes took place both directly (Pl. XXVII, fig. 7) and through the stomata. A detailed discussion of the method of penetration is given by Nishikado and Miyake (9) for the Japanese strain. In this inoculation work, the leaves were inclosed in 15-inch test tubes and held in position by means of cotton. The transpiration water was sufficient to cause the germination of the conidia.

Rice flowers, from the booting period to the stage when the lemma and palea closed after the extrusion of the stamens, were painted with conidial suspension in sterile distilled water. A camel's-hair brush was dipped in sterile distilled water and rubbed on the surface of each of the petri dishes containing cultures of the Japanese strain JNS<sub>1</sub> and the Louisiana strain



AIS of *H. oryzae*. A total blasting resulted of the panicles painted with the spore suspension. The complete browning of all parts where the brush had come in contact took place in 24 hours (Pl. XXIV, B, 1 and 2). The checks

TABLE 2. Summary of Inoculation Work on Rice<sup>7</sup> through Different Stages of its Development, Conducted during the Summer and Autumn of 1921

Varieties Used	Parts of Rice Inoculated	Age of Plant	Results	
			Inoculated	Checks
7369 F4 Macan.....	Entire seedlings	20 days	+	0
7369 F4 Macan.....	Entire seedlings	28 days	+	0
5891 F6 Binicol.....	Entire seedlings	20 days	+	0
5891 F6 Binicol.....	Entire seedlings	28 days	+	0
5891 F6 Binicol.....	26 culms	70 days	+	0
5891 F6 Binicol.....	35 culms	127 days	+	0
7328 F2 Roxas.....	1 stool	137 days	+	0
7328 F2 Roxas.....	100 grains in each of 12 pots, inoculated at time of planting	.....	+	0
1643 Honduras.....	Entire seedlings	5 days	+	0
..... Iray.....	Entire seedlings	5 days	+	0
6040 F6 Kinagaykay....	Entire seedlings	5 days	+	0
7369 F4 Macan.....	Entire seedlings	5 days	+	0
3052 F5 Inintiw.....	Entire seedlings	5 days	+	0
4496 F6 Maliro.....	Entire seedlings	5 days	+	0
10231 F2 Binangbang....	Entire seedlings	5 days	+	0
5896 F Ganado.....	Entire seedlings	5 days	+	0
5895 F3 Binalayang....	Entire seedlings	5 days	+	0
5891 F6 Binicol.....	Entire seedlings	5 days	+	0
7328 F2 Roxas.....	Entire seedlings	5 days	+	0
..... Sinampaga.....	41 culms at booting stage	135 days	+	0
..... Bonguet.....	33 culms at booting stage	135 days	+	0
5891 F6 Binicol.....	5 panicles in boots	.....	+	0
5891 F6 Binicol.....	2 stools	202 days	+	0
4496 F6 Maliro.....	2 panicles in boots	.....	+	0
4496 F6 Maliro.....	12 fertile heads	202 days	+	0
9118 Tiniaong.....	Seedlings	10 days	+	0
9047 Kinandang kinapal..	Seedlings	10 days	+	0
4495 F5 Putyucanon....	Seedlings	10 days	+	0
91112 Lubuang.....	Seedlings	10 days	+	0
17565 Daluson.....	Seedlings	10 days	+	0
17566 Kinastila.....	Seedlings	10 days	+	0
7365 Inasimang.....	Seedlings	10 days	+	0
17567 Murmuray.....	Seedlings	10 days	+	0
9115 F Dinalaga.....	Seedlings	10 days	+	0

painted with sterile distilled water produced healthy caryopses (Pl. XXIV, B, 3).

Injecting conidial suspensions of the Louisiana cultures AO and AUR into the flowers at the opening time also produced total blasting of the injected flowers. Complete browning also occurred in 24 hours. For injecting spore

<sup>7</sup> The varieties of rice used in these experiments, with the exception of 1643 Honduras, were obtained from the Department of Agronomy, University of the Philippines, Los Baños. Variety 1643 Honduras was obtained from the Crowley Rice Station, Louisiana. The numbers before the Philippine varieties are accession numbers, and the letter "F" indicates the generation in the Philippines.



suspensions, the small tip of the medicine dropper was drawn to a capillary size. Care was taken not to touch any part of the flower with the needle-like point of the dropper. The checks did not turn brown, even at harvesting time, and no fungus developed when plated out.

The results of all inoculation work show that the Louisiana and the Philippine strains of *H. oryzae* are parasitic on all parts of the rice above ground, and even to some extent on the roots. The results obtained with the Louisiana strain at Madison confirmed those found in the Philippines in 1918 and 1919. Sprouting rice is readily infected, causing death of the young plants, and such infection is probably responsible in part for the irregular stand in seed-beds and in the field. The experiments also show that rice may be infected by the fungus during its entire life history. The Philippine and the Louisiana strains of *H. oryzae* compare in pathogenicity with the Japanese cultures of the same species when used in artificial inoculation work. The pathogenicity of the Japanese *H. oryzae* on different parts of rice during the entire life history of the plant in Japan has been reported by Hori in his original description in 1901 (17), and by Nishikado and Miyake (9) in 1922.

Artificial cross-inoculation experiments on various species of grasses have shown that *H. oryzae* is capable of producing lesions on plants other than rice. Of 31 species belonging to 23 genera of grasses which the writer used in July, 1922 (11), 23 species representing 17 genera have not been reported by Nishikado and Miyake (9). All of these were infected, and showed lesions when examined 36 hours after inoculation. Some of the species had more lesions than others. The species not mentioned by Japanese workers are: *Agrostis palustris* Huds., *A. stolonifera* L., *A. tenuis* Sibth., *Andropogon caricosus* L., *A. rufus*, *Aristida longiseta* Steud., *Avena sativa* L., *Bromus inermis* Leyss., *B. unioloides* H., B., & K., *Dactylis glomerata* L., *Danthonia semiannularis* R. Br., *Euchlaena mexicana* Schrad., *Festuca pratensis* Huds., *Holcus sorghum sudanensis* (Piper) Hitchc., *Lolium subulatum*, *Oryzopsis miliaceae*, *Panicum decompositum* R. Br., *Paspalum dilatatum* Poir., *P. laranagai*, *Pennisetum villosum* R. Br., *P. ruppelii* Steud., *Poa compressa* L., and *Secale cereale* L. The seedlings used in the inoculation work were 40 days old. 27-day-old potato-dextrose-agar cultures of both the Japanese JNS1 and the Louisiana AIS strains were used.

## CONTROL MEASURES

### Sanitation

Since the fungus is capable of living as a semi-saprophyte in the soil, it is advisable to remove all infected plants and destroy them by burning. From artificial cross-inoculation work it seems probable that the fungus can live over winter on other grasses. Therefore, if the disease is once established in a locality, it is likely to persist in the field indefinitely.

### Resistant Strains of Rice

The writer noted in 1918 and 1919 in the Philippines that different varieties of rice exhibit different degrees of infection as shown by laboratory experiments and field observations (10). The difference in degree of infection was also observed in Louisiana in September, 1922. No work, however, was done to determine the varying degrees of resistance of the different varieties of rice, or to find whether these apparent differences in susceptibility are temporary or lasting.

### Temperature Relations of the Rice, the Fungus, and the Development of the Disease

In the present work, the writer attempted to determine whether the behavior of the fungus and that of the rice were the same at different temperatures, in order to suggest a practical method of control. A more detailed account of these experiments is given in a later paper by the writer.

Considering the results obtained, it seems possible to reduce the infection of the seedlings by *Helminthosporium oryzae* at 36° C., and to some extent at 32° C. At 36° C., however, rice seedlings gradually turn yellow if allowed to remain too long at this temperature. At 36° C., there is sometimes a breaking down of the green coloring matter of the leaves of the seedlings, and tip-burn often occurs. At 28° C. and 32° C., the development of the lesions from primary infection is very rapid, due perhaps to the rapid growth of the fungus and of the rice seedlings at these temperatures. Only occasional blighting at emergence was observed at 32° C. and at 36° C. Since rice sprouts in from two to three days at these temperatures with very light infection, infected rice may be started in seed beds with a temperature of about 36° C. After emergence, the temperature may be lowered to from 28° C. to 32° C., at which temperatures the subsequent growth of the seedlings is best.

It seems possible to reduce infection from *H. oryzae* over-wintering in the soil and in crop residues of heavily infected fields by flooding the soil with water to a depth of about 10 centimeters. The writer found that certain Philippine lowland varieties of rice will germinate under 10 to 20 centimeters of water provided the temperature is 24° C. to 28° C. With this amount of water over the soil, the conidia did not germinate to produce infection, while lowland varieties of rice emerged through the water in from two to three weeks. With 20 centimeters of water, however, the seedlings produced were spindling.

### Hot-Water Seed Treatment

Nishikado and Miyake (7, 8) in Japan adopted the hot-water seed treatment for the control of *Helminthosporium oryzae*. These authors state that the hot-water treatment does not absolutely control the disease, but reduces it considerably.

### Chemical Treatment

In 1922, Nishikado and Miyake (9) state that solutions of copper sulfate, corrosive sublimate, silver nitrate, calcium hypochloride, formaldehyde, and phenol may serve for disinfecting rice on account of the high germicidal actions of these solutions on the conidia of *Helminthosporium oryzae*. Experiments conducted by the writer in the Philippines in 1918 and 1919 and at Madison, Wisconsin, in 1920, using rice heavily infected with seed-borne mycelium, showed almost as many blighted seedlings in the treated lots as in the untreated lots after treatment with 1:1000 mercuric-bichlorid solution. Unhulled rice in these experiments was treated with the solution from 15 to 60 minutes and hulled rice from 5 to 10 minutes, and the grains were washed with five changes of sterile distilled water before they were plated out or planted in sterile soil. Infection takes place from the seed-borne mycelium, which is not reached by the action of the bichlorid of mercury.

### CONCLUSIONS

The evidence heretofore presented leads to the conclusion that the *Helminthosporium* disease of rice, as it occurs in Java, Sumatra, Japan, India, Italy, the Philippine Islands, and in Louisiana, at least, of the southern United States, is caused by the same species of fungus, *H. oryzae*. In the Straits and Federated Malay States and in southern China, a similar disease occurs of which the causative *Helminthosporium* may be this same species, but the identity of this is yet to be determined.

In connection with the causative agent, the writer noted that there are certain morphological as well as physiological differences between the different strains of *H. oryzae* occurring in various rice-growing districts of the world. According to Breda de Haan, the Javanese strain has fusiform conidia. The Japanese strain has obclavate or fusiform spores, while the Philippine material has fusoid to obclavate, and the Louisiana fungus, fusoid, fusoid-cylindrical, or obclavate to clavate spores.

The writer has not determined to what extent climatic conditions affect the variations of the shape and size of the spores of *H. oryzae* under field conditions in the Philippines and in Louisiana. The effect of weather conditions prevalent during growth and conidium-production upon the size and shape of the conidia was not determined. What effect the growth of the fungus upon other members of the grass family might have upon the shape, size, color, and behavior of the spores was not studied. Different specimens collected at different times showed a wide range in shape and size.

Studies of the effect of various plant tissues upon the production of the perfect stage of *H. oryzae* showed that both the Philippine and the Louisiana strains produced only the conidial or imperfect stage. The fungus did not produce any other spore forms upon the various plant tissues, or upon infected parts of rice, even after exposure out of doors during the winter

season. In consequence perhaps of the semi-saprophytic habit of *H. oryzae*, and of its ability to grow on other members of the Gramineae, it is able to persist in the field even if rice is not grown. Inasmuch as rice is a tropical or subtropical crop, there are no adverse weather conditions for which *H. oryzae* should develop spores more resistant and well protected in order to persist. As far as known, *H. oryzae* does not produce the ascigerous stage. Whether or not the fungus produces the perfect stage in nature, or whether it may be induced to form this stage in artificial culture, is yet to be determined.

In artificial cultures on potato-dextrose agar in Van Tieghem cells, and on potato-dextrose agar smears on slides, the Japanese strain produced conidia earlier than the Louisiana strain. All successive stages of the development were the same.

On dilute glycerin added to water agar, the Louisiana and the Japanese strains were alike in regard to spore-production. Only the sporulating cultures of these two strains produced conidia. The Philippine non-sporulating culture produced conidia on all the glycerin agars. The coloration produced on the medium by the Japanese and by the Philippine strains was the same.

The Philippine and the Louisiana strains produced conidia on some plant tissues. While the Louisiana strain produced sporulating cultures on the cotton at the bottom of the test tubes containing sterilized plant tissues, the Philippine strain did not.

The Helminthosporium disease of rice varies somewhat in symptoms and markedly in severity in the different regions in which it occurs. These variations are attributable to differences in climatic conditions. In Japan, distortion of infected plants and blasting of panicles of rice are shown by the specimens from Mr. Nishikado (Pl. XXII, C, 2). Specimens of panicles of rice from Mr. M. Mitra of Pusa, India, were totally blasted and sterile (Pl. XXII, C, 4). In India, Sundararaman (16) also reports heavy damage to the rice during heading time on account of heavy rains. Only a few kernels on infected rice panicles from Java, Sumatra, Louisiana, and the Philippine Islands (Pl. XXII, C, 2, 3, 5, 6) show a conspicuous fungous growth.

In this work, microscopic examination of both sides of the palea and lemma as well as of the ovary wall showed that the fungus is carried in the seed from one season to another as dormant mycelium. On account of its occurrence on the ovary walls of rice grains, treatment with chemical solutions does not affect the fungous mycelium within the rice hulls. By treating the grains with chemical solutions, either of higher concentration or of weaker concentration for a longer period, to insure penetration into the fungous mycelium on the ovary wall, the germination of the rice is considerably reduced. Work has been done to determine what temperatures of the soil would hasten germination of rice in order to outgrow the disease

and to avoid the most serious form of the malady. The results showed that, although rice and *H. oryzae* have almost the same temperature requirements, the production of the disease is more severe at temperatures below the optimum for both. The results further indicate that seeding during the warm part of the year will avoid the most severe form of the disease. Starting the seedlings in hot beds at temperatures of about 36° C., before the seedlings are transferred to temperatures of 28° C. to 32° C., will materially reduce infection from seed-borne mycelium or from the fungus in the soil or in crop residues.

Under field conditions, certain varieties of rice planted side by side show differences in the degree of infection by *H. oryzae*. Whether these apparent differences in degree of susceptibility are lasting or only temporary is yet to be determined. According to observations, however, there is a possibility of developing varieties of rice highly resistant to the disease, if not totally immune.

#### SUMMARY

The results obtained in the present work may be summarized as follows:

1. The Helminthosporium disease of rice occurs in Java, Sumatra, Japan, China, India, Straits and Federated Malay States, Italy, the Philippine Islands, and in Louisiana in the southern United States.
2. The severity of the disease varies in different places, probably on account of climatic conditions. In the Philippines, the losses among seedlings may be 10 to 58 percent. In Louisiana no actual count was made. From the writer's observations, the disease occurs on all varieties of rice in culture there, although it is practically unrecognized.
3. The disease occurs as leaf-spots on seedlings and older plants, as seedling- and leaf-blight, as infections of culms and sheaths, and to some extent as infection of the glume. The disease causes total blasting of kernels in cases of severe infection of the panicles. In all cases, many of the apparently healthy grains carry the mycelium. When the invaded parts of the rice die, sporulation takes place and conidia are spread by the wind, causing secondary infections.
4. The Helminthosporium disease of rice in the Philippines and in Louisiana is caused by *Helminthosporium oryzae* Breda de Haan. The different strains have certain morphological and physiological differences.
5. The conidia are borne apically and on knee-like projections on the aërial conidiophores. The conidiophores are borne singly or in fascicles. The conidia germinate readily from one or both ends; broken spores germinate from the sound and broken ends. The conidiophores may also germinate readily. Penetration of germ tubes into rice tissues may be direct or through natural openings.
6. The fungus sporulates sparingly on artificial media. Frequently sporulation may be secured on plant tissues, and especially on the cotton which has been bathed in the decoction of the plant tissue used. Occasional-

ly sporulating cultures may be produced. On different carbohydrates and on glycerin agars, only the sporulating cultures of the Japanese and the Louisiana strains of *H. oryzae* produced conidia. The Philippine non-sporulating culture produced conidia on glycerin agar.

7. *Helminthosporium oryzae* grows best at 28° C. The maximum temperature is about 40° C., and the minimum is about 16° C. The exact temperatures for the maximum and minimum were not determined.

8. The fungus may be induced to sporulate on ordinary 1.5-percent potato-dextrose agar by irritation of the mycelium and by partial desiccation either in the sun or in artificial light above the optimum temperature for growth.

9. The best vegetative development on potato-dextrose agar was obtained at a pH-concentration of 8.6–8.8, and only bare growth of the mycelium was obtained at a pH-concentration of 2.4–2.6. The maximum alkalinity of the medium which will allow the fungus to grow was not determined in this work.

10. The fungus overwinters as dormant mycelium on the palea and lemma and on the ovary walls of the grains, where the hyphae are attached by means of star-shaped appressoria. These hyphae are responsible for the greater part of the primary infection in the field.

11. As far as known, *H. oryzae* does not produce the perfect stage. Conidia are produced throughout the rice-growing season. The conidia are carried by the wind, causing secondary infection on all parts of the rice plant during its entire life history, that is, from seedling to the maturity of the grains. The fungus is pathogenic not only to rice but also to various members of the grass family.

12. On account of the semi-saprophytic habit of the fungus, properly carried out sanitary measures will aid in reducing the sources of infection in the field.

13. It seems probable that certain varieties of rice are more resistant to the disease than others. The extent and duration of this apparent difference in degree of susceptibility remains an open question.

14. It seems possible to reduce infection from the organism overwintering in the soil and in the crop residue by seeding in soil at a temperature of about 36° C., or by starting seedlings in seed-beds at this temperature. At 36° C., rice germinates in two to three days after planting with very light infection, but this temperature is not suitable for the subsequent development of the seedlings. At 32° C. rice germinates as fast as at 36° C., and the temperature is around the optimum for the subsequent development of the seedlings. Occasional blighting of the seedlings, however, occurs at 32° C. The growth and development of the lesions at 32° C. is very rapid, perhaps because of the rapid growth of both the fungus and the rice at this temperature.

15. As to the fungus overwintering in the soil and crop residues, it seems



probable that infection can be prevented by submerging the soil in water to a depth of about ten centimeters, provided the temperature of the water is 24° C. to 28° C. Lowland varieties of rice emerged through water 10 to 20 centimeters deep at these temperatures, and the conidia mixed with the soil at planting or suspended in the water did not produce infection.

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## DESCRIPTION OF PLATES

## PLATE XXII

Rice seedlings showing the early stage of the disease. Note the brown and oblong spots on the leaves, leaf sheaths, and roots of the seedlings. On the leaf sheaths some of the spots run together, forming elongated brown areas with light-colored centers.

A. Twelve-day-old seedlings of 91112 Lubuang germinated on June 18, 1922, from naturally infected kernels of the Philippine rice, showing the brown and oblong spots on the leaves, leaf sheaths, and roots.

B. Six-day-old infection on sheaths and leaves of rice grown to maturity in the greenhouse during the summer of 1921. Inoculation was made by atomizing the leaves and sheaths with conidial suspension. On 70-day-old plants, notice the oblong brown spots on leaves and sheaths. The result shows that leaves and sheaths of plants about to flower are as easily affected as younger plants.

C. Panicles of rice from various countries, showing the symptoms of the disease from field materials and the varying degrees of severity in different localities. (1) Brown and blasted panicles of rice from Sumatra; courtesy of Dr. R. D. Rands. The effect upon the kernels is similar to that in artificial inoculation experiments upon young flowers in the greenhouse. This panicle shows infection while very young. (2) Totally distorted panicle of rice before it emerges from the boot; a condition caused by the fungus in Japan; courtesy of Mr. Y. Nishikado. The distorted panicle is covered with a black mass of conidia and conidiophores. The specimens of rice panicles received from Mr. Nishikado were totally distorted in consequence of the attack of the fungus. From these specimens it seems probable that the disease is much more severe in Japan than it is in the Philippines, Sumatra, Java, India, and Louisiana. This may be due to climatic conditions. (3) A portion of a panicle of rice collected in Java; courtesy of Dr. Rands. Note the kernel near the top, completely covered with a black mass of sporophores. Two other grains on the lower part are also covered with the sporophores. The symptoms on the kernels of Javanese rice are much like those produced on the rice in Louisiana and in the Philippines. (4) Totally blasted panicles of rice from India; from specimen given by Dr. A. G. Johnson. This specimen was received by Dr. Johnson from Mr. M. Mitra of Pusa, India. Mr. Mitra determined the fungus as *Helminthosporium oryzae*. (5) Specimen of a rice panicle collected by the writer at Crowley, Louisiana, September 18, 1922. On this large panicle only five kernels, three of which are shown in the picture, were covered with the black, velvety mass of conidia and conidiophores. The affected kernels are much like those of the rice collected in Java and the Philippines. (6) A panicle of Philippine rice infected by the fungus from material received from Mr. G. M. Reyes of the Bureau of Science, Manila. This shows the browning of the palea and lemma and the presence of conidia and conidiophores on the discolored areas of the kernels.

## PLATE XXIII

A. Symptoms of the *Helminthosporium* disease on older rice leaves from field infections. (1) Leaves of Louisiana rice, showing numerous small circular, oblong, or elongate spots. The older spots have light-colored centers. This is a young stage of infection on leaves of mature plants collected by the writer in Louisiana in September, 1922. (2) Leaves of rice from the Philippines, collected by Mr. Reyes, showing much larger spots elliptical in shape, several running together to form larger ones. This is an older and more severe infection on the leaves of mature plants. (3) Leaves of Japanese rice, showing the oblong spots only slightly smaller than those on the Philippine rice; courtesy of Mr. Nishikado.

B. Lesions on the sheaths of the rice variety Watari-bune from Louisiana. (1) Infected with the Japanese culture JNS1 sprayed with conidial suspension on the sheaths and culms at heading time. (2) Infected with the Louisiana culture AIS sprayed in the same manner. From greenhouse infection experiments August 7, 1922. Specimens collected

September 24, 1922. Both the Japanese culture JNSr and the Louisiana culture AIS produced similar symptoms. The brown spots have less definite margins than those on the leaves. Note the running together of the brown areas on the sheaths. Sheaths of mature plants are as readily attacked by *H. oryzae* as are those of seedlings.

## PLATE XXIV

A. Four-day-old infection from the Louisiana culture AIS of *H. oryzae* of flowers of variety 5891 F6 Binicol (1 and 2), and on leaves and stems of the variety 7328 F2 Roxas (3), showing the brown oblong spots on the rice flowers and on leaves and sheaths. From artificial inoculation experiments on greenhouse-grown plants during the summer of 1921, using conidial suspension in distilled water applied by means of an atomizer. The result shows that flowers, leaves, and sheaths of rice are easily attacked by *H. oryzae*.

B. Artificially infected heads of variety 1643 Honduras in greenhouse experiments of August 7, 1922. (1) Painted with the conidia of the Louisiana culture AIS by means of a camel's-hair brush. (2) Painted with the conidia of the Japanese culture JNSr by means of a camel's-hair brush. Note the browned and blasted kernels of the entire panicle. (3) Check showing healthy grains of normal color. The experiment showed that the presence of abundant conidia outside the palea and lemma of rice flowers produces total blasting. All panicles were harvested September 24, 1922.

## PLATE XXV

70-hour growth of the Louisiana culture AIS at temperatures from 20° C. to 40° C. on potato-dextrose agar. As explained in the text, the Philippine and the Japanese cultures made similar relative growths. At 20° C., there was a small growth with conidia. At 24° C., the growth was larger with abundant conidia. At 28° C., the growth was much larger with abundant conidia. This was the best temperature for the growth of the culture. At 32° C., the growth was not as large as that at 24° C. and was thinner. At 36° C., the growth was only slightly larger than at 20° C., and at 40° C. there was very little growth.

## PLATE XXVI

Conidia of *Helminthosporium oryzae*: (1, *po*) Philippine strain; (2, *al*) Louisiana strain; (3, *ju*) Javanese strain; (4, *jp*) Japanese strain. Mounted in 10-percent caustic-potash solution. Drawn with the aid of a camera lucida, using a Leitz microscope, ocular 4, objective 6. All drawn to the same scale.

Conidiophores of the four strains of *H. oryzae*: (1, *po*) Philippine strain; (2, *al*) Louisiana strain; (3, *ju*) Javanese strain; (4, *jp*) Japanese strain. Mounted in 10-percent caustic-potash solution. Drawn with the aid of a camera lucida, using a Leitz microscope, ocular 4, objective 6. Drawn to the same scale as the conidia.

## PLATE XXVII

FIG. 1. Mycelium on ovary wall of rice grain, obtained by soaking rice grains in 10-percent sodium hydroxid for 21 hours.

FIG. 2. Fusion of cells of adjacent hyphae of the Japanese culture JNSr in Van Tieghem cells. Drawn with the aid of a camera lucida, using Bausch and Lomb microscope, ocular 2, objective 4 mm.

FIG. 3. Germinating sound and broken conidia of the Philippine strain of *H. oryzae*, five hours old.

FIG. 4. Germinating sound and broken conidia of the Louisiana strain, 19 hours old. Mounted in sterile distilled water. Drawn with the aid of a camera lucida, using Bausch and Lomb microscope, ocular 2, objective 4 mm.

FIG. 5. Production of conidia by the Japanese culture JNS1 in Van Tieghem cells containing dilute potato-dextrose agar 24 hours old. Same magnification as figures 2, 8, and 9.

FIG. 6. Mycelium on the palea of rice grains obtained by soaking the grains in 10-percent solution of sodium hydroxid for 21 hours.

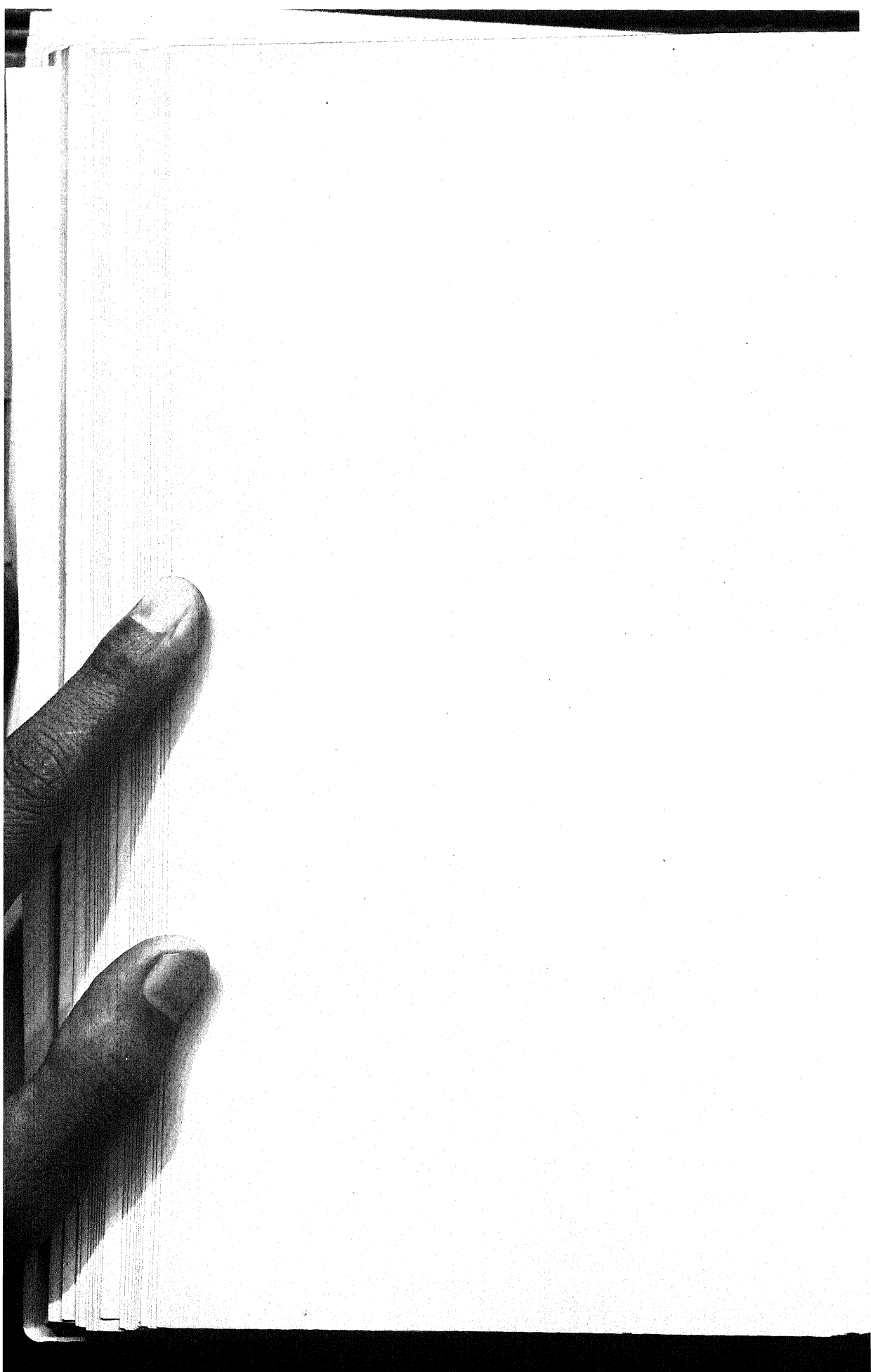
FIG. 7. Direct penetration by a germ tube of *H. oryzae* of leaves of mature plants artificially inoculated in the greenhouse. Figures 1, 6, and 7 were drawn with the aid of a camera lucida using a Leitz microscope, ocular 4, objective 6.

FIG. 8. Production of conidia by the Louisiana strain AIS in Van Tieghem cells with dilute potato-dextrose agar as in figure 5, 48 hours old.

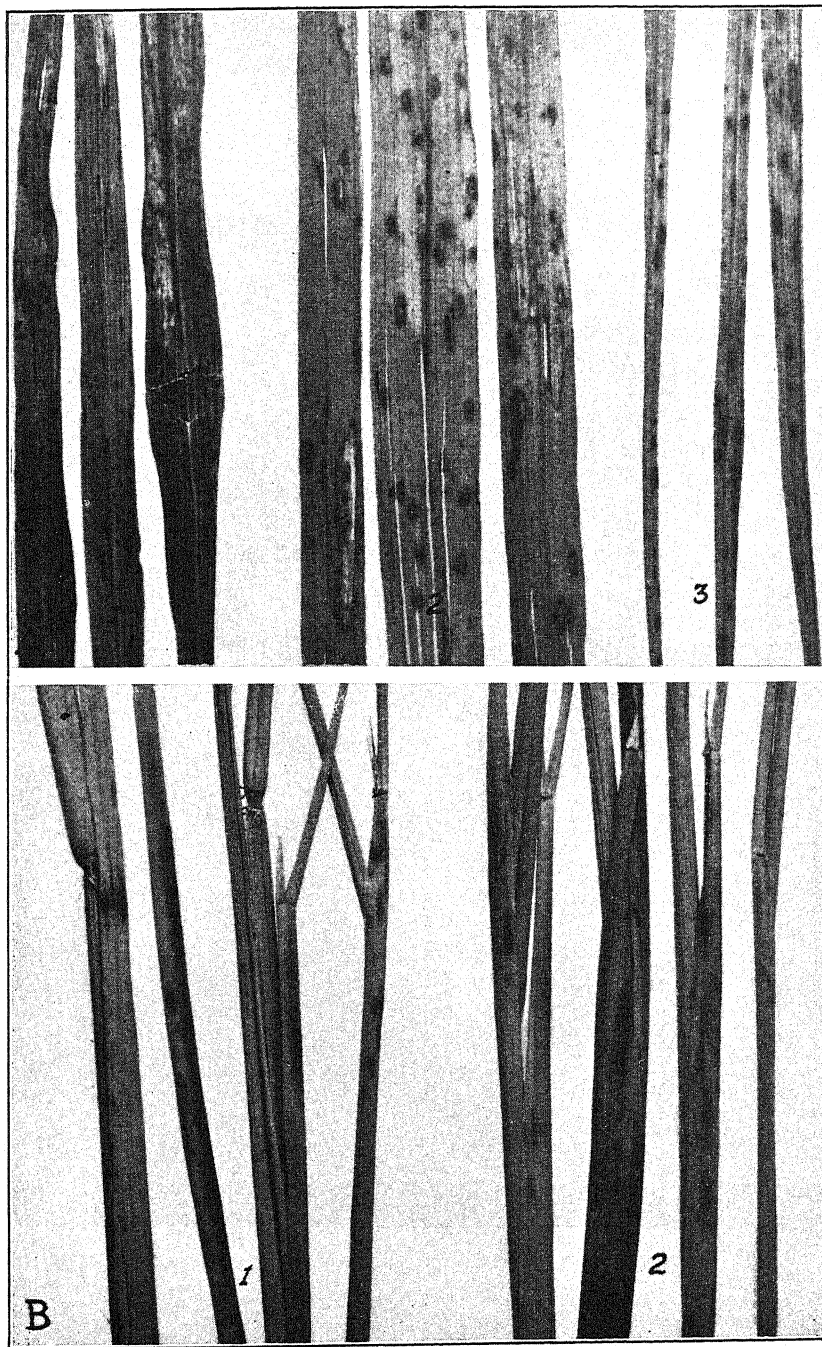
FIG. 9. Fusion of cells of adjacent hyphae as in figure 2. Drawn with the aid of a camera lucida, using Bausch and Lomb microscope, ocular 2, objective 4 mm.



OCFEMIA: HELMINTHOSPORIUM DISEASE OF RICE



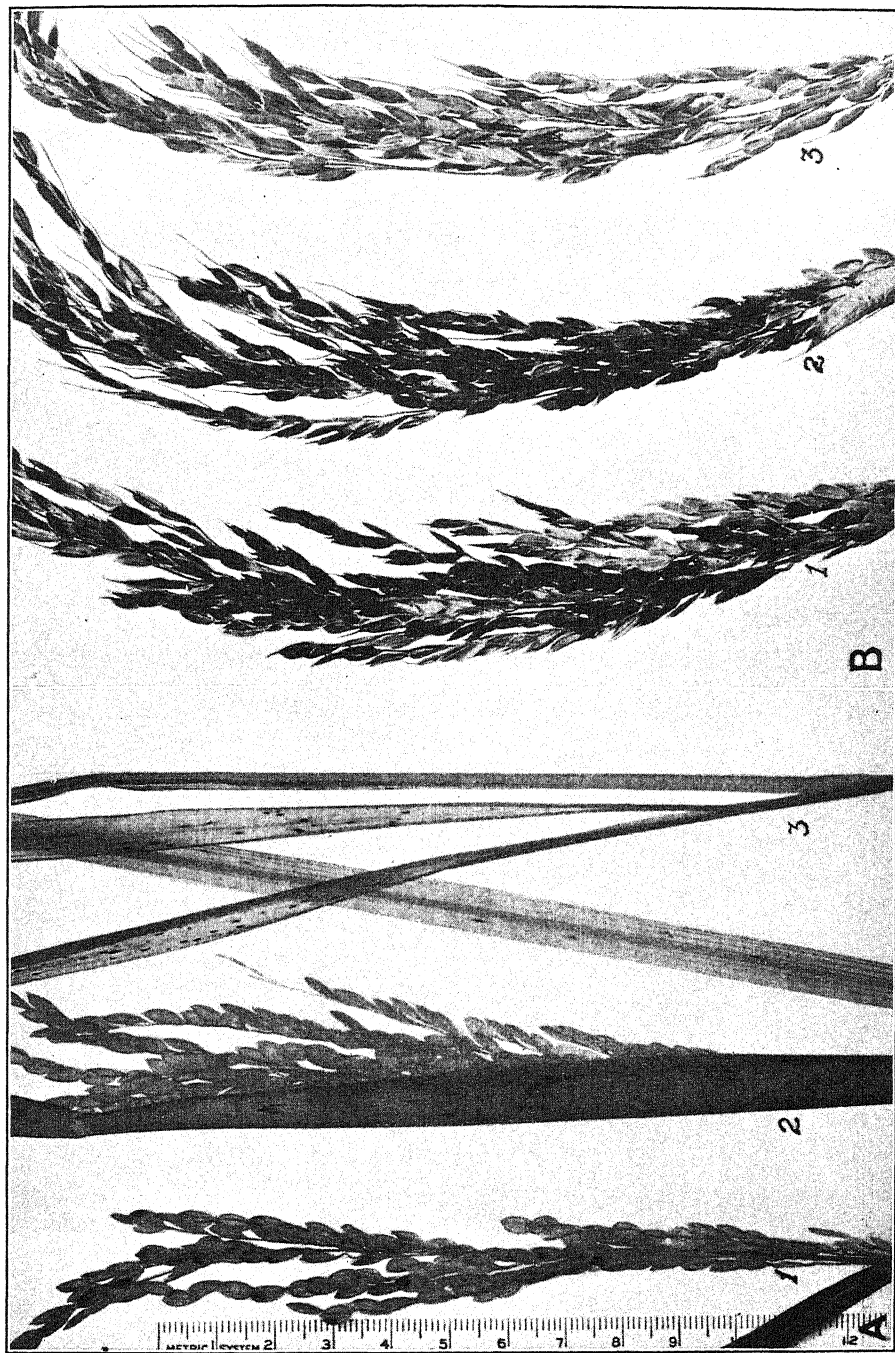




OCFEMIA: HELMINTHOSPORIUM DISEASE OF RICE

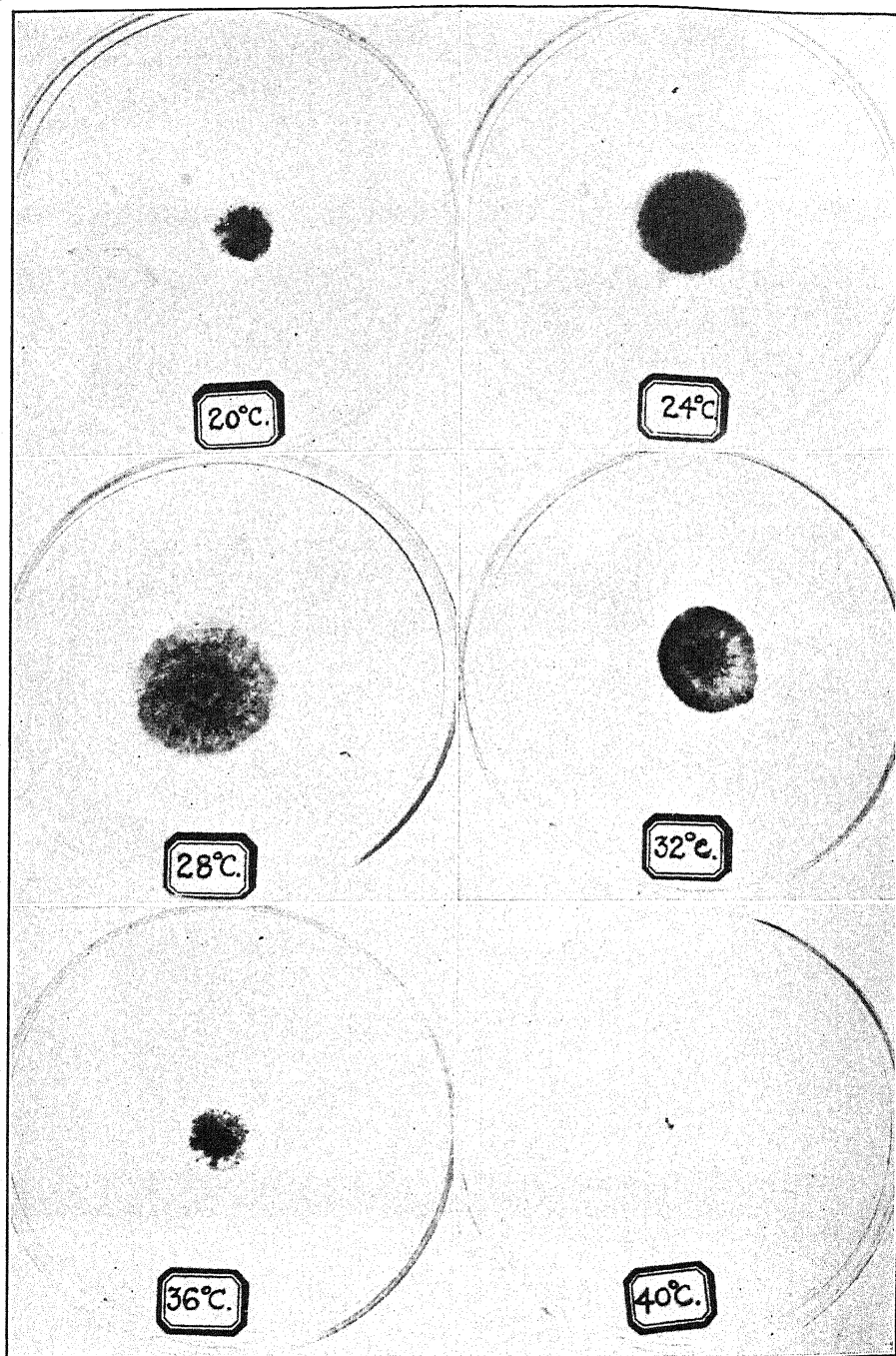






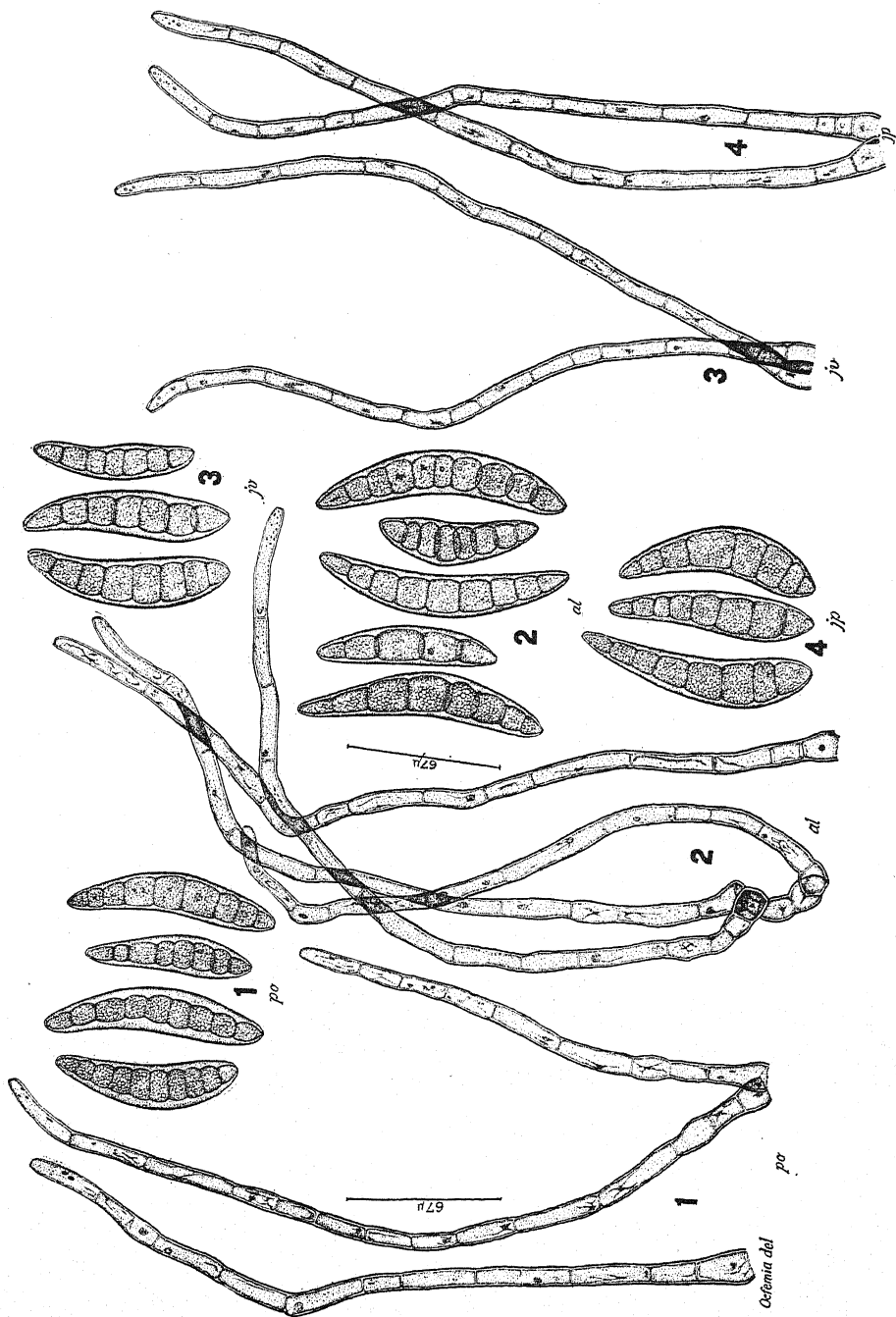
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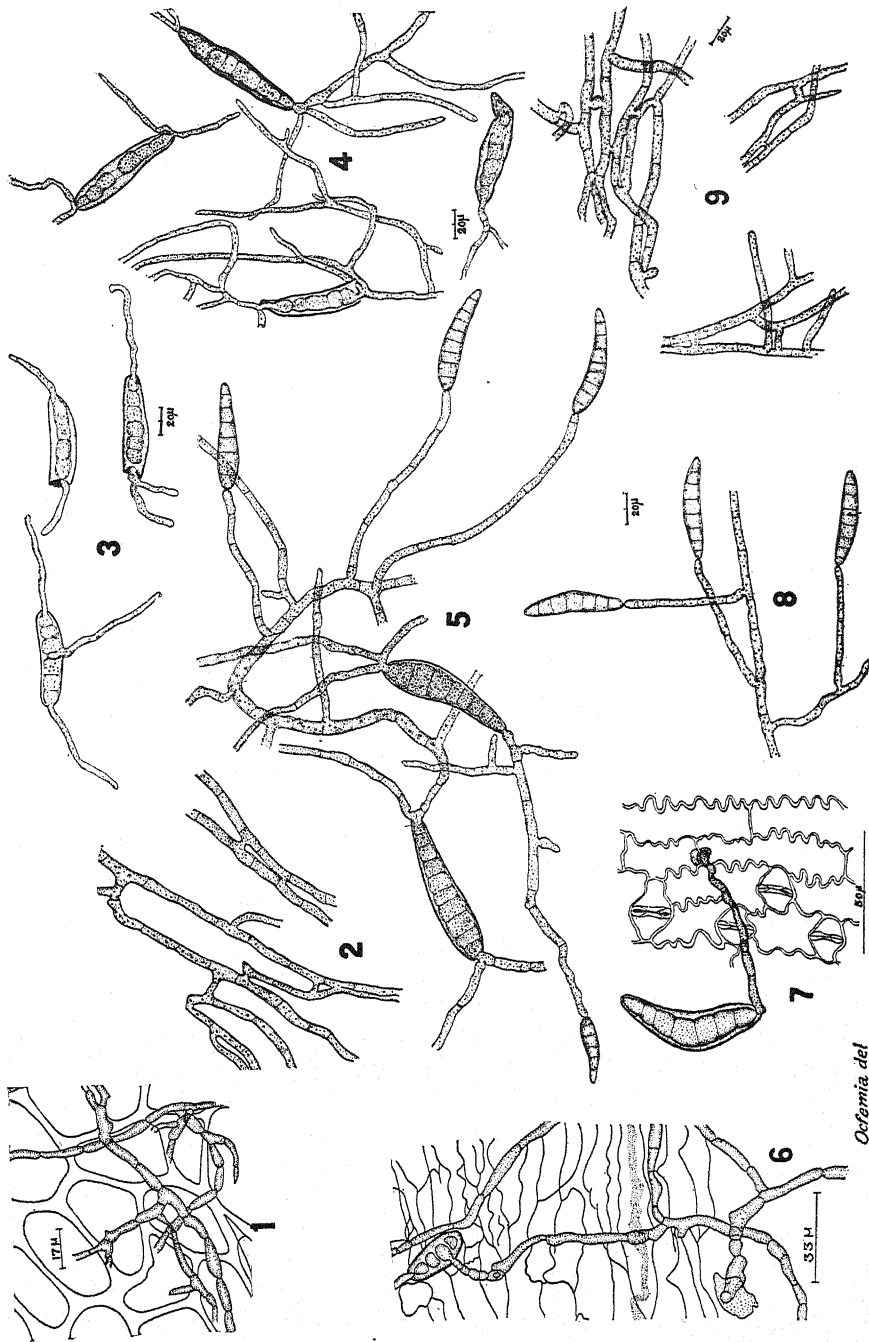




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## THE PLANT FORMATIONS ON THE CORAL REEFS ALONG THE NORTHERN COAST OF CUBA

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Cuba and many other islands of the West Indies are surrounded to a considerable extent by coral reefs. These are very characteristic along many parts of the northern coast of Cuba and the small adjacent islands and keys. They form hard, very uneven, and rough masses of stones along the shore and at considerable distances from the coast. The writer has made his observations from June until September, and has repeatedly visited several places in the northern parts of various provinces of the island.

The vegetation, which has been but little investigated, is quite uniform throughout the entire region.

At many places, the coral reefs are very little elevated above the level of the sea; consequently the rock at distances of three to five meters from the shore is constantly moistened by the water from the sea. On the other hand, however, there are at certain places higher reefs, usually of older formation, which may stand from two to thirty and sometimes more meters above sea level.

The reefs are mainly formed by the following coral polyps: *Millepora alcicornis* L., *Platygyra viridis* Les., *Isopora muricata* L., *Agaricia Cailletii* Duch. and Mich., *Porites porites* Pall., *P. asterioides* Lam., and a few other species. Analyses of coral reefs have shown that they are mainly composed of the following compounds:  $\text{SiO}_2$ , 0.23 percent;  $\text{Al}_2\text{O}_3$ , a trace;  $\text{FeO}_3$ , a trace;  $\text{CaO}$ , 55.16 percent;  $\text{MgO}$ , 0.2 percent;  $\text{H}_2\text{O}$ , 0.54 percent;  $\text{CO}_2$ , 43.74 percent.

Plant growth at such places is very interesting. Most plants are not only halophytic, but have adapted themselves to a strongly calcareous environment. There are various species of the land flora which are the first settlers, and which are succeeded by entirely other formations and associations when the islands become older and more removed from the sea, after secondary soil types such as humus have been formed.

At first the algal flora may be considered; this however, is still imperfectly known. Many characteristic species of algae grow between the tide marks; others occur somewhat more removed from the land, and still other species grow only at considerable depths. The species most frequently encountered are certain Chlorophyceae, of which *Rhizoclonium tortuosum* Kuetz. forms fleecy masses in tide pools and on other algae between the tide marks on the somewhat exposed shore. *R. riparium* (Roth) Harv. and a

few other species of this genus also form roundish patches of a few millimeters to half a decimeter in diameter, growing on the reefs in similar localities. They are often the only individuals on the highest tide marks where usually no other species are to be found, with the exception of some poorly developed plants of Dictyopteris. On the other hand, tide pools may contain a few other species. These pools may cover a surface of from a few square decimeters to five or six or more square meters; they continually contain water, which is now and then renewed by sea water splashing over it and keeping it moving. During low tide in these pools we observe, besides species of Rhizoclonium, also the interesting *Padina Durvillaei* Borg.

The cliffs extending from about two decimeters to one meter below the surface of the water contain several Chlorophyceae, Phaeophyceae, and Rhodophyceae. Most plants hardly reach a length of more than one decimeter. Some parts of the cliffs are entirely covered by a pure formation of Dictyopteris; at other places we see *Padina Durvillaei* Borg. or *Laurencia obtusa* Lamour. Most common, however, are the associations composed of a large number of species, especially *Galaxaura cylindrica* Soland, *Eucheuna isiforme* (Ag.) J. Ag., *Codiolum longipes* Fosl., *Chondria tenuissima* (Good. and Woods.) Ag., species of Corallina, Nemalion, Chantransia, Ulva, and others. Where the sea is rather shallow, one may observe this very interesting association of short algae covering an extent of several hectares.

Where the sea becomes considerably deeper, other formations and associations of algae occur, composed of other species which frequently are of considerable length. They belong mostly to the Rhodophyceae and Phaeophyceae, although the already described shorter species are also present. I regret that it was technically impossible to study the algal conditions at farther distances from the Cuban coast and at a greater depth.

The unicellular flora, belonging partly to the plankton, and including also species found among the larger algae, is mainly composed of representatives of the following genera: Campylodiscus, Bacillaria, Pleurosigma, Navicula, Actinocyclus, Actinoptychus, and Coscinodiscus among the diatoms, and several dinoflagellates.

The seed plants on the rocky shore which take first possession of the land include but a few species. They are, however, very characteristic. At a distance of from one to six meters from the shore, the thick-leaved *Sesuvium microphyllum* Willd. and *Phylloxerus (Gomphrena) vermicularis* (L.) R. Br. are conspicuous. The former has thick oval to roundish leaves, whereas those of the latter species are more elongated.

It is especially important to examine from a physiological standpoint the anatomical structure, which may be compared with that of species of other regions in similar localities but under different climatic conditions. The succulent leaves of *Sesuvium* contain a small-celled epidermis, covered by a thin cuticle, many of the epidermal cells containing anthocyan in

their vacuoles. This tissue is followed by a well developed palisade parenchyma; the cells of the first layer are short and roundish, whereas those of the three succeeding layers are elongated. The palisade parenchyma is followed by a well developed water-containing tissue, whose cells are large and contain a large amount of storage water and slime. Quite different is the anatomy of the creeping stem. Its epidermis is covered by a thick cuticle and is underlaid by round cortical cells, which contain but few chloroplasts. It is apparent that the elements of the xylem are very narrow.

*Sesuvium microphyllum* grows on the reefs where little or no soil has accumulated. Its roots penetrate the fine pores of the corals and fasten tightly about them. Roots form abundantly at every node of the stem. It frequently happens that parts of the stem are broken off by the waves and are moved elsewhere. Such pieces act as natural cuttings; they easily root and soon cover the rocks.

*Phylloxerus (Gomphrena) vermicularis* (L.) R. Br. is, in growth and behavior, somewhat similar in appearance to the species just mentioned and, on many rocks, is as abundant, although it does not produce roots as readily at the nodes of its creeping stem. Both species are strongly halophytic.

Where sufficient soil and sand have accumulated between the rocks which are exposed to the sea water, one finds considerable formations of *Salicornia perennis* Mill. and of *Distichlis spicata* (L.) Greene. Intermingled with the former one may encounter some individuals of *Sesuvium* or of *Phylloxerus*, but, where grass is well developed, it is rare to find other plants. In the struggle for life the grass soon predominates, and it is difficult for other species to gain foothold between the small, but very tough, root-stocks. These species are also often found in the tide pools, which are partly dry during high tide.

*Sesuvium*, *Phylloxerus*, and *Distichlis* do not root deeply into the soil, whereas the roots of *Salicornia* penetrate to a depth of from one to four decimeters. In some other places *Salicornia Bigelovii* Torr. is abundant, although it is more frequently found in large saline marshes.

The succulent stem of *Salicornia perennis* contains large epidermal cells, which are surmounted by a relatively thick cuticle. This tissue is succeeded by a palisade parenchyma composed of from one to three layers. It is only in this tissue that photosynthesis can take place, since the actual leaves are very rudimentary as in all species of *Salicornia*. Between these cells appear at different intervals large colorless cells; these contain no chloroplasts and their walls have internal ring and spiral thickenings. The form of these cells is cylindric, sometimes pointed toward the base. They are tracheids in which a considerable amount of water is stored. The rest of the cortex is composed of very large cylindric or round cells, containing much water. Toward the fibro-vascular bundle these cells become smaller. The inner wall of the endodermis is very thick.

As these plants are under the direct influence of the sea water, it will be useful to analyze the content of the oceanic water. Its content is as follows: O, 85.79 percent; H, 10.67 percent; Cl, 2.07 percent; Na, 1.14 percent; Mg, 0.14 percent; Ca, 0.05 percent; K, 0.04 percent; S, 0.09 percent; Br, 0.008 percent; C, 0.002 percent. Other elements are contained in sea water but only in minute traces, none of them reaching 0.001 percent. Considering the oceanic salts, we find that the following amounts are available: NaCl, 77.76 percent;  $MgCl_2$ , 10.8 percent;  $MgO_4$ , 4.74 percent;  $CaSO_4$ , 3.60 percent;  $K_2SO_4$ , 2.46 percent;  $MgBr_2$ , 0.22 percent;  $CaCO_3$ , 0.34 percent.

Where the reefs become a little higher, other species predominate, among which *Conocarpus erecta* L. (Combretaceae) is first apparent. It grows close to the shore, but where sea water never reaches the plants except during storms. At such places it is a very low shrub, hardly reaching one decimeter in height; farther from the actual reefs, however, the individuals become much taller, especially in the coastal mud. This species has leathery leaves oval to elliptic in shape, which have a strongly developed palisade parenchyma, a spongy parenchyma being practically absent. The species may cover the coral rocks over large areas. Often it is associated with *Borrchia arborescens* (L.) DC. (Compositae). This shrub reaches a height of from 30 centimeters to 1.20 meters; it has whitish, succulent, rather large leaves, and yellow inflorescences. Pure formations of this species are also by no means rare, although they are often to be found at some distance from the shore. A cross section of a leaf shows apparently a compound epidermis. While the leaf is very young the epidermis becomes two-layered, the upper layer of cells forming appressed hairs which give the whitish color to the plant. Between the two layers of cells is a very thick membrane. The palisade parenchyma is normally developed, and the tissue used for storage of water constitutes a large part of the leaf.

Another species regularly found in the same localities is *Rachicallis maritima* (Jacq.) Schum., a low shrub with very small leaves and yellow flowers. It belongs to the Rubiaceae. The crooked twigs lie close against the rock. Infrequently, one finds a pure formation of this species. Its anatomy is of much importance, especially that of the small leaves which are perfectly adapted to a xerophytic environment. The epidermis is composed of very small cells which are covered above by a thick cuticle. Directly beneath the epidermis is a parenchyma used for the storage of water. Its cells are large, and small intercellular spaces appear between them. Next follows a tissue resembling a palisade parenchyma composed of small cells. The leaves are rolled upward on both sides. In the hollow thus formed, the epidermis is quite different; instead of having a thick cuticle, it develops long one-celled hairs which prevent, to a considerable extent, transpiration through the stomata in the hollows. Furthermore, that part of the leaf whose epidermis carries the hairs has pushed through a

small opening where the leaf is folded (Pl. XXVIII, fig. 1). The midrib, on the lower surface of the leaf, has much the same anatomical construction as the upper surface; that is, the epidermis has a thick cuticle and is followed by a water-storage tissue. The fibro-vascular bundle is very small, the vessels being exceedingly narrow.

Several plants, especially *Sesuvium*, *Phylloxerus*, *Salicornia*, and *Borrichia*, have a very salty taste, which suggests a high concentration of sodium chlorid in the cells, and consequently a high osmotic pressure. This osmotic pressure was measured by the writer in studying plasmolysis of various tissues of different species. Potassium nitrate was used as a cause of plasmolysis. Solutions were used of strengths from 1 percent to 10 percent.

From the following table it is evident that the osmotic pressure differs somewhat in various tissues as well as between different individuals of the same species, which may be expected as a matter of individual fluctuation.

In table I only two examples of each species are recorded, although more observations have been made.

TABLE I. *Osmotic Observations in Different Species of Plants Growing on Coral Reefs and in the Immediate Vicinity of the Sea*

Name	Tissue Studied	Percent KNO <sub>3</sub> Causing Plasmolysis at 22° C.
<i>Salicornia perennis</i>	1.... Palisade parenchyma.....	6
	Water tracheids.....	5.6
	Water tissue.....	5.6
	Cortex of roots.....	6.2
	2.... Palisade parenchyma.....	5.8
	Water tracheids.....	5.3
	Water tissue.....	5.4
	Cortex of root.....	6.1
<i>Sesuvium microphyllum</i>	1.... Epidermis.....	8.4
	Palisade parenchyma of leaves.....	8.8
	Palisade parenchyma of leaves.....	8.8
	Cortex of stem.....	8.6
	Cortex of root.....	8.8
	2.... Epidermis.....	7.9
	Palisade parenchyma of leaves.....	8
	Cortex of stem.....	8.1
<i>Borrichia arborescens</i>	Cortex of root.....	8
	1.... Palisade parenchyma of leaves.....	5.2
	Water tissue.....	5.2
	Cortex of root.....	5.2
	2.... Palisade parenchyma of leaves.....	5.1
	Water tissue.....	5.2
	Cortex of root.....	5.2



In the experiments above reported, observations were recorded at a time when some cells of a certain tissue began to plasmolyze whereas others had not entirely reached that stage. This may give a good average for each tissue observed.

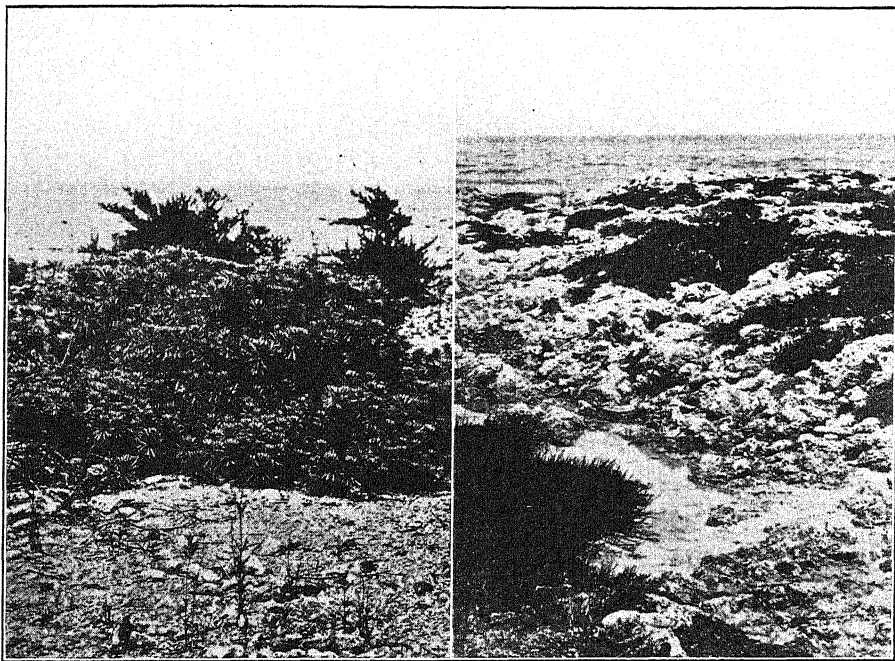
At a distance of 20 to 30 meters from the shore, a considerable amount of soil and sand has accumulated between the coral reefs; in fact, at many places the rock has disappeared from the surface. The influence of the sea water, excepting at times of heavy storms, is here *nil*. Here we find extensive thickets of *Conocarpus erectus*, the plants having here a totally different appearance; instead of the branches lying directly against the soil or rock, they are erect, forming little trees or, more usually, shrubs. *Borrchia arborescens*, *Rachicallis maritima*, and *Sesuvium portulacastrum* L. are also abundant, whereas *Salicornia perennis* has disappeared entirely, and of *Distichlis spicata* but few individuals are left. Other species appear. *Flaveria linearis* Lag. is here a constant associate. It is a low-growing herbaceous plant, usually decumbent, and covering large areas. *Ipomoea pescaprae* (L.) Roth creeps over a considerable area, covering much of the sandy soil. Other plants also become more common, but many of them are to be found farther inland. Among these I mention only *Flaveria trinerva* (Spreng.) Mohr., *Tribulus cistoides* L., *Kallstroemia maxima* (L.) T. & G., *Crotalaria pumila* Ort., *Cracca hispidula* (Pers.) Kuntze, *Chloris barbata* (L.) Nash, and *Melanthera deltoidea* Mich.

Along other parts of the shore, other species are abundant, often presenting a pure association of different species of *Euphorbia* (Chamaesyce); e. g., *E. buxifolium* Lam., *E. Blodgettii* Engelm., *E. prostrata* Ait., *E. thymifolia* Burm., *E. trichotoma* HBK., and *E. Torrabasi* Urb. They creep on or between the rocks but are never found in close proximity to the shore, as is, for example, *Sesuvium microphyllum*. Sometimes three or four different species of *Euphorbia* grow near each other. In some instances, other species of other families abound. I need but mention *Cuphea Parsonsia* (L.) R. Br., *Lippia nodiflora* L., *L. canescens* Kth.; they grow only on the soil between the rocks. In other but similar situations, we encounter *Ageratum maritimum* HBK., *A. muticum* L., *Chenopodium ambrosioides* L., *Iresine flavescens* HBK., *I. celosioides* L., *Aschyranthes maritimus* (St., Hill) Stand. *Batis maritimus* L., which is also to be found in such places, is more abundant in saline marshes and similar localities.

Very characteristic is *Heliotropium humifusum* HBK., probably endemic. It is a low and very compact herbaceous plant, much-branched, although it reaches a height of only 1 to 2 centimeters. It has the appearance of many alpine plants like *Erytrichium* and some species of *Androsace*. Other species of *Heliotropium* also occur, having an entirely different habitat; for example, *H. curassavicum* L., an annual with glaucous linear leaves and branches, and *H. parviflorum* L., with broader leaves, which also grows on waste grounds.



Very typical is *Tournefortia gnaphaloides* R. Br., a member of the Boraginaceae with hairy lanceolate leaves. This shrub reaches a height of 4 to 12 decimeters and frequently forms dense clumps on the coastal rock at a little distance from the shore. Often this species is accompanied by *Suriana maritima* L., belonging to the Simarubaceae and possessing leaves similar to those of the previously named species, although the shrub has a much looser and more uneven shape. In such localities we also often encounter *Stachytarpheta jamaicensis* Vahl., *S. cayamensis* Vahl.,



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TEXT FIG. 1. *Tournefortia gnaphaloides*, *Suriana maritima*, and *Iresine celosioides* on coral reefs along the coast of northern Cuba.

TEXT FIG. 2. *Conocarpus erectus*, *Borrichia arborescens*, *Salicornia perennis*, and *Sesuvium microphyllum* on coral reefs along the coast of northern Cuba.

*Canavalia obtusifolia* (Lam.) DC., *Tridax procumbens* L., *Iresine celosioides* L., *Heliotropium humifusum*, *Cakile lanceolata* (Willd.) Schultz—although the last-named species is more common near the sandy shore—and *Tourneria ulmifolia* Gris. Except where there is a considerable amount of soil between the rocks, the plants are, as a rule, a considerable distance from each other.

On some parts of the coast there is a shrub association composed of *Suriana maritima*, *Rachicallis maritima*, *Borrichia arborescens*, *Tournefortia gnaphaloides*, and *Coccolobis wifera* (L.) Jacq.; soon *Coccolobis* becomes the predominating factor, and at last composes pure formations which

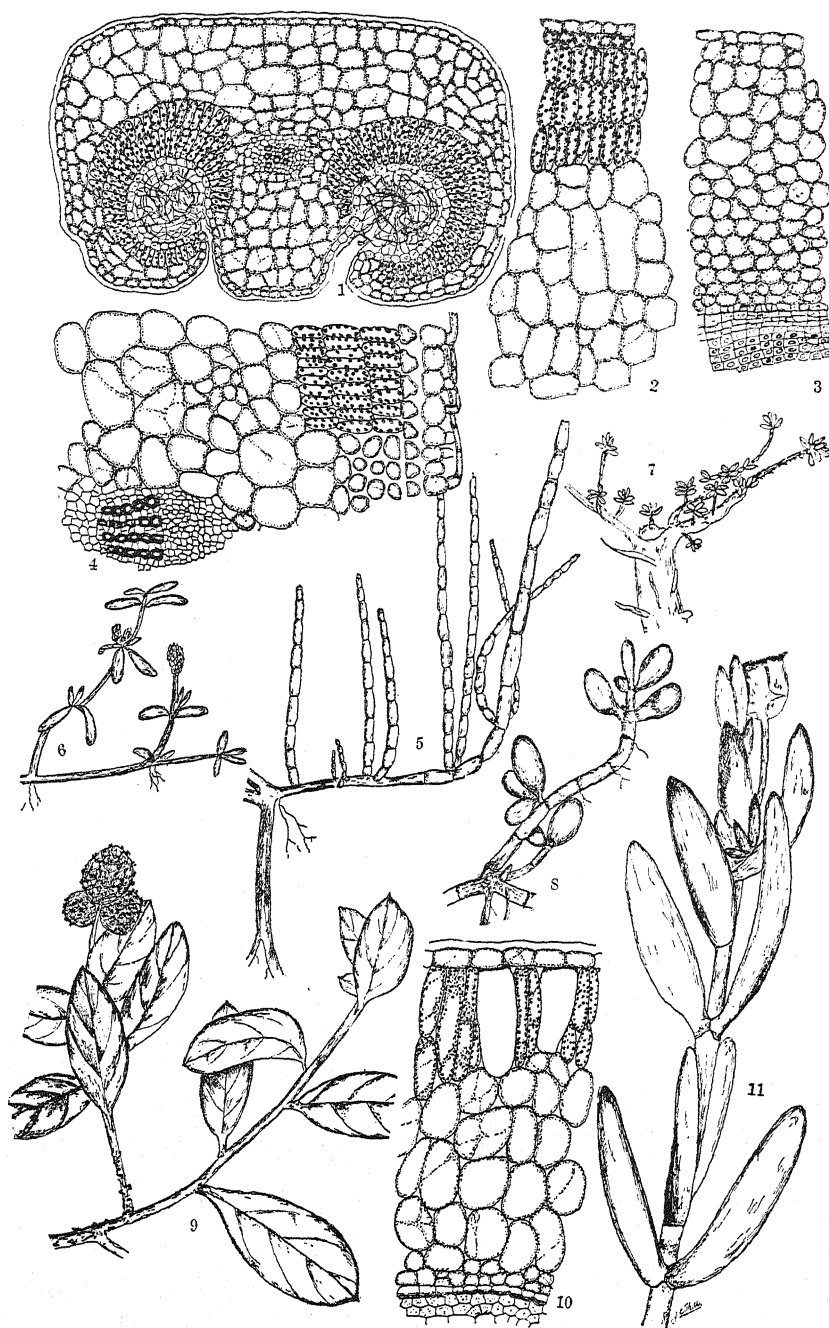
extend for several kilometers along the coast. This formation often makes dense thickets which are less difficult to enter than are some of the thickets of a mixed nature.

Behind the *Coccolobis* formation we often find suddenly a shrubbery extending for several hundreds of hectares, which is composed of a large number of different species, most of which are also found farther inland on the rocky hillsides.

In such thickets we find *Cassia occidentalis* L., *Lantana aculeata* L., *Rauwolfia ternifolia* HBK., *Tecoma stans* (L.) HBK., *Jatropha gossypifolia* (L.) Pohl., *Euphorbia* (*Arthothamnus*) *cassythoides* (Bois.) Millsp., *Omphalia diandra* L., *Plumiera sericifolia* C. Wright, *Cestrum diurnum* L., *Hamelia patens* Jacq., *Fagaria fagaria* (L.) Sarg., *Duranta plumieri* Jacq., *Callicarpa dentata* Jacq., *Comoclada dentata* Jacq., and a few cactus species. Between these, *Serjania subdensata* Juss., *Echitis umbellata* Jacq., and *E. neuroides* Gris. are the most common vines. There is absolutely no species which predominates and forms a distinct association; they all grow intermingled in great profusion. Where the reefs rise from 5 to 10 meters above sea level, one finds, against the slopes of the almost vertical reefs, practically only *Sesuvium microphyllum* and *Phylloxerus vermicularis*. As soon as the limit is reached at which the rock becomes horizontal, these two species remain within a few meters, but soon *Conocarpus erectus* and *Rachicallis maritima* abound. Both species are very low here, though sufficiently removed from the sea water. Very soon a formation is found of *Opuntia tuna*, which often becomes very dense; after this species other shrubs occur, especially *Plumeria marginata* Gris., *P. sericifolia* Wright, *Omphalia diandra* L., *Euphorbia cassythoides*, with cylindric succulent stems and very rudimentary leaves; *Fagaria fagaria*, species of *Caesalpinia*, *Coccolobis uvifera*, and the above-mentioned vines which occur in the dense thickets. The shrubbery near the coast, however, is not as dense as the one described following the *Coccolobis* formation, but at about 30 to 40 meters from the shore this low shrub formation gradually emerges into the very dense thickets.

#### EXPLANATION OF PLATE XXVIII

- FIG. 1. Cross section of a leaf of *Rachicallis maritima*.
- FIG. 2. Cross section of a leaf of *Sesuvium microphyllum*.
- FIG. 3. Cross section of a stem of *Sesuvium microphyllum*.
- FIG. 4. Cross section of a leaf of *Borrchia arborescens*.
- FIG. 5. *Salicornia perennis*.
- FIG. 6. *Phylloxerus vermicularis*.
- FIG. 7. *Rachicallis maritima*.
- FIG. 8. *Sesuvium microphyllum*.
- FIG. 9. *Conocarpus erectus*.
- FIG. 10. Section of a stem of *Salicornia perennis*.
- FIG. 11. *Borrchia arborescens*.



UPHOF: PLANT FORMATIONS ON CORAL REEFS



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## THE PHYSIOLOGY OF POLLEN

### IV. CHEMOTROPISM; EFFECTS ON GROWTH OF GROUPING GRAINS; FORMATION AND FUNCTION OF CALLOSE PLUGS; SUMMARY AND CONCLUSIONS<sup>1</sup>

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#### CHEMOTROPISM

In seeking an explanation of the direction of pollen-tube growth in the pistil of the plant, three different possibilities suggest themselves. The tube may be passively guided from the receptive surface to the micropyle of the ovule by certain anatomical features of the tissue traversed; it may be oriented in its course by the diffusion from particular centers of substances having a chemotropic effect; or, when growing over free surfaces, the pollen tube may arrive at the micropyle purely as a matter of chance.

A rather extensive literature pertaining to this subject has grown up, concerning especially the tropistic reactions of pollen tubes. This we shall review briefly. Van Tieghem (1869) regarded hydrotropism as the cause of the pollen tube's entering the stylar tissue. Working with a large number of plants, but particularly with *Torenia*, Strasburger (1878) found no evidence of chemotropism for ovules. Pollen tubes growing in drop cultures showed no tendency in the presence of ovules to grow into the micropyles more frequently than in any other direction, and usually, upon coming in contact with ovules, grew over them and separated from them on the other side. In search of an explanation of the incompatibility of matings in heterostyled plants, Correns (1889) found no difference in chemotropic reaction between the pollen from the two forms of *Primula acaulis*, nor indeed was any chemotropism demonstrated in this species.

Molisch (1893) reported that the pollen tubes of *Narcissus tazetta* are negatively aërotropic. When cultured in sugar solutions under a cover glass, only the grains in the peripheral region germinated and these sent their tubes inward toward the region of lower oxygen tension. Precautions

<sup>1</sup> Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

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were taken to prevent evaporation at the edge of the cover glass. Molisch states, however, that this phenomenon was not general. In the same species it was found that the stigma, parts of the petiole and flower, the micropyle and funiculus of the ovule, and stigmas from other species exerted a chemotropic effect on pollen tubes growing in their vicinity. Molisch concluded that in *Narcissus* the female sex apparatus through definite secretions calls forth a chemotropic response in the pollen tube which leads it to the egg.

Miyoshi (1894) regarded chemotropism for the stigma as a general phenomenon. He reports having seen drops of liquid at the micropyle which he believed were secretions that attracted the tubes. Some sugar, possibly sucrose, was thought to be the active agent. It was demonstrated that pollen tubes are indifferent to light and that geotropism plays no rôle in determining the direction of their growth. On cutting off the style of *Digitalis purpurea* and pollinating the cut end, it was observed that some tubes grew into the air, whereas others grew in the reverse direction and their tips appeared ultimately at the tip end. If the stigma was cut off or damaged, the tubes grew and projected still more. These results eliminated chemotropic phenomena as possible causes of orientation in the style and led Miyoshi to the conclusion that pollen tubes follow the line of least resistance to the ovary. In the ovarian cavity, however, a chemotropic stimulation accounts for their entry into the micropyle.

Lidforss (1899 a) confirmed Molisch's observation that *Narcissus* tubes cultured on 5-10 percent sugar-gelatin media grow toward the stigma. He did not believe, however, that the active substance was a carbohydrate; nor could he demonstrate any chemotropic effect toward organic acids, such as formic, acetic, lactic, succinic, malic, tartaric, or citric acid. A bit of onion root exercised a strong chemotropic effect, which fact indicated to the author that the active substance was widely distributed. Diastase, even after boiling or treatment with sulphuric acid, exerted a pronounced chemotropic effect, suggesting to Lidforss that some protein substance was concerned. Dialyzed albumin attracted *Fritillaria* tubes. Protein derivatives, such as tyrosin, were without effect. Lidforss believed that the chemotropic effect of these protein substances probably plays an important rôle in the passage of the pollen tube through the style. Kirkwood (1906) considered the embryo sac the source of a stimulant of some sort, probably a sugar, by which the pollen tube is directed unerringly toward it.

Tokugawa (1914), following Lidforss, divided pollen into two groups, namely, "saccharochemotropische" and "proteochemotropische." It was shown that *Narcissus* pollen tubes were attracted to the stigmas of *Prunus* and *vice versa*, but that often this reciprocal relation did not hold. Working with *Nicotiana*, East and Park (1918) found no certain evidence of positive chemotropism for parts of the gynoeceum. Knowlton (1922) observed that the pollen tubes of *Antirrhinum* growing on artificial media grew toward and even penetrated stigmatic tissue placed on the culture. Pollen tubes



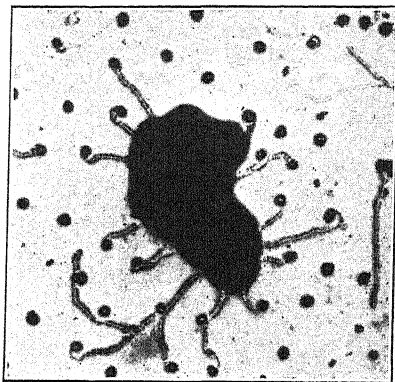
of apple were similarly attracted to the stigma of the apple, but in this form the phenomenon was less marked.

Anatomical studies led Capus (1878) to the view that no particular arrangement exists which facilitates the entrance of the pollen tube into the micropyle and that some undetermined physiological phenomenon causes the tube to be attracted to its objective. Dalmer (1880), on the other hand, quotes Behrens (1875) as having found that the long walls of the stylar cells are easily separated from each other while the cross walls possess a marked tenacity. Where a stylar canal was present, Behrens found that its outer layer had secretory functions. Dalmer expressed the view that the distribution of the conducting tissue in the ovary depends wholly on the position of the micropyle. In *Polygonum* and *Daphne*, where the micropyle lies directly at the base of the style, no conducting tissue is developed. In forms where the micropyle is not so conveniently situated, it was found that the surfaces leading to it are covered with papillose cells. Dalmer dissents from Capus' view that some physiological phenomenon must be invoked to explain the passage of the tube to the micropyle, and holds that in the carefully and fully studied cases the conducting tissue has been found to extend to the micropyle. The pollen tube, in his opinion, is forced by outside conditions to enter the micropyle. Guéguen (1901), after an extensive study of the anatomy of the style, concluded that in monocotyledons the distribution of the conducting tissue is entirely dependent upon variations in number and position of the ovules. It is in the Liliaceae that the greatest simplicity of conducting apparatus is found. Here stigma passes to stylar canal and stylar canal to the conducting tissue of the ovary walls and placenta very gradually. Among the dicotyledons the conducting tissues show greater variability and complexity, but of all the regions traversed by the pollen tube after its emission on the stigma, the ovary presents the least variation in the structure of the conducting apparatus. In the great majority of cases, the rôle of conduction devolves upon the epidermis of the internal face of the carpellary leaf or on the epidermis of the placenta. Guéguen investigated the subject from the phylogenetic point of view and gave comparatively little attention to the physiological considerations involved.

During the course of the author's investigation, the various forms of pollen used have been tested for their chemotropic reactions to various plant tissues, principally gynoecium parts. In only two species, *Antirrhinum* and the paper-white *Narcissus*, was the phenomenon of chemotropism demonstrated. Text figures 1, 2, and 3 show pollen tubes of *Narcissus* growing toward placenta, epidermis, and ovule respectively of the same form on a culture medium consisting of 2-percent agar, 15-percent cane sugar, and a little sterile yeast. Pollen tubes of *Antirrhinum* were also found to be attracted toward their own stigmas. In *Hippeastrum*, *Scilla*, *Nicotiana*, *Cucumis*, *Primula*, *Lythrum*, and *Vinca*, pollen tubes growing on artificial media showed no tropistic reaction to gynoecium parts.

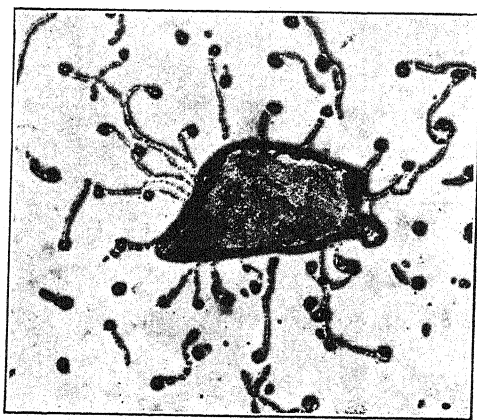


In conjunction with these tests for chemotropism to bits of plant tissue, experiments were made to determine if the pollen tubes of the various species were attracted by cane sugar or by sterile yeast. Agar plates were prepared



TEXT FIG. 1. Pollen tubes of paper-white *Narcissus* grown on an artificial medium, showing chemotropism for placental tissue.

carrying varying proportions of sugar and yeast, and small sections of the semi-solid medium were cut from these in such a way that two blocks each derived from a different medium could be fitted together so that

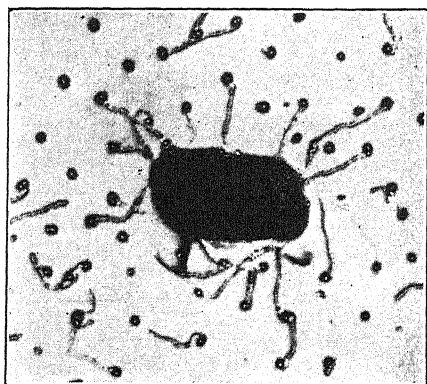


TEXT FIG. 2. Paper-white *Narcissus* pollen tubes grown on an artificial medium, showing chemotropism for epidermal tissue from the internal wall of the ovary.

their upper surfaces were practically continuous. Pollen was distributed with a needle on either side of the junction of the two blocks, and the direction the tubes took during growth on the various combinations of blocks was noted. As was expected, germination of all the species tested

was not secured on each medium. In a considerable number of cases, however, good growth on both blocks of the pair was secured. On pairs of blocks containing the respective concentrations of sugar, 0-3, 3-10, 0-10, 0-20, 3-20, and 10-20, the pollen tubes of *Cucumis*, *Primula*, *Hippeastrum*, and *Nicotiana* grew entirely at random. Differences in concentration of sugar in the adjacent areas occasioned no orientation of the tubes. Similarly, on pairs in which the differential was sterile yeast, the tubes appeared to grow entirely at random.

Using Pfeffer's method of fine capillary tubes filled with the solution being tested and closed at one end with paraffin to retard diffusion, marked chemotropism of *Narcissus* pollen tubes for sodium malate has been found. Further experiments are in progress with salts of malic acid.



TEXT FIG. 3. Pollen tubes of paper-white *Narcissus*, showing chemotropism for an ovule on an artificial medium.

It is interesting to note, in connection with text figures 1, 2, and 3, that the pollen tubes of *Narcissus* are attracted not only by the ovules but also by the placenta and by pieces of the epidermis from the internal wall of the carpel. A quantitative method of determining the relative chemotropic values of these different tissues has not yet been devised, but it is quite obvious that, unless the ovule exerts a greater attraction to pollen tubes in the ovarian cavity than other parts, chemotropism can play no rôle in directing the pollen tube to the micropyle.

#### THE EFFECTS ON GROWTH OF GROUPING GRAINS AND THE PROBLEM OF SELF-STERILITY

Early in our work it was observed that tubes arising from groups of grains on artificial media were longer than those produced by single isolated grains. Attention was first directed to the condition in cultures of *Nicotiana*, where it regularly appeared that the longest tubes came from large

masses of pollen grains. Casual observations on other forms indicated a similar situation and it was decided to put the matter to careful experimental test. Cucumis pollen was chosen as giving high germination and as being of sufficient size to be quickly and conveniently plated as required. The method of testing was simple. The groups, consisting of four grains in a cluster, were arranged on one side of the culture and spaced to avoid overlapping of the tubes of adjacent groups during growth. On the same culture, parallel to the series of groups but remote from them, lines of single grains were similarly arranged. The tubes were measured when elongation had ceased.

A greater amount of manipulation with the needle was required to plate the single grains than the quartets, and it was at first thought that perhaps a difference in the amount of injury effected was responsible for the difference in growth. It was found, however, that such was not the case. Groups composed of grains previously singled out still gave greater growth.

TABLE I. *Summary of the Results of 5 Tests on the Relative Amounts of Growth from Pollen Grains Spaced Singly and from Groups of 4 on Agar Cultures Containing Cane Sugar (Lengths in Microns)*

Test	Singles		Quartets		Difference
	No. of tubes Measured	Average Length	No. of tubes Measured	Average Length	
1	91	343	95	466	123
2	50	338	104	563	225
3	126	323	91	476	153
4	120	502	119	659	157
5	69	261	70	410	149
Average	456	366	479	529	163

In table I the results of five tests on the relative amounts of growth from single grains and from groups of four on agar cultures containing only cane sugar are set forth. In each test the average growth from the quartets of pollen grains exceeded that from the singles; the differences range from 123  $\mu$  to 325  $\mu$ . The average difference, considering all the tubes measured in each series, is 163  $\mu$ , or an increase in growth due to grouping of 44.5 percent.

The results of six similar tests on the same medium with sterile yeast added are given in table 2. Here the results are somewhat more variable, but the growth from the quartets is on the average clearly in excess. In test 4, the average length of the tubes in the series of singles exceeded that in the quartets by 236  $\mu$ , a result we can not account for. The series of tests on yeast media involved the measurement of 1063 tubes, approximately equally divided between singles and quartets. The tubes from the latter

averaged 255  $\mu$  longer than those from the single grains, a 34.6-percent increase in growth attributable to grouping.

TABLE 2. *Summary of Results of 6 Tests on the Relative Amounts of Growth from Pollen Grains Spaced Singly and from Groups of 4 on Agar Cultures Containing Cane Sugar and a Little Sterile Yeast (Lengths in Microns)*

Test	Singles		Quartets		Difference
	No. of Tubes	Average Length	No. of Tubes	Average Length	
1	136	522	95	563	41
2	65	466	88	947	481
3	59	707	81	1331	624
4	91	1270	111	1034	-236
5	71	932	121	1254	322
6	78	758	67	1055	297
Average	500	768	563	1034	266

Groups of four grains each were used largely as a matter of convenience; with larger groups, the accurate determination of the length of each tube was more difficult. It was found, however, that with some forms groups of four did not give an appreciably greater average amount of growth than grains spaced singly. Very probably the small size of the pollen grains in these species and the rather rangy growth of their narrow tubes accounts for this, since in such cases if the size of the group was increased the effect due to grouping was unmistakably shown. None of the forms tried failed to respond under such conditions, although, with such species as *Lythrum*, groups of less than ten grains failed to give a measurable effect.

In order to determine if the substance or substances contributing to increased growth in groups of tubes were present as such in the pollen grains, quantities of fresh pollen were removed from anthers and after being crushed in a mortar were scattered on the agar plates either somewhat uniformly over a narrow strip or in small heaps fairly uniform in size. Single whole grains were placed at regular intervals among these, and the

TABLE 3. *Summary of the Results of Experiments to Determine if Pollen Tubes Give Greater Growth in the Presence of Crushed Pollen Grains (Lengths in Microns)*

Test	Without Crushed Grains		With Crushed Grains		Difference
	No. of Tubes	Average Length	No. of Tubes	Average Length	
1	39	1387	39	1434	+ 47
2	49	824	33	502	-322
3	20	609	22	809	+200
4	87	404	68	734	+330
Average	195	727	162	865	+138

amount of growth they gave was compared with that of single pollen tubes growing on portions of the same plate free from the crushed material. The results of four tests with *Cucumis* pollen are given in table 3.

In three of the four tests reported in table 3, the tubes growing among crushed pollen grains gave a greater average growth than those on the plain medium. In the other test the latter tubes were longer. Little significance can be attached to these results, however, for it was found upon careful examination that the method used in preparing the crushed pollen was faulty; in the process of crushing many grains were not broken, as revealed by their ability to swell when placed on the culture and to germinate in some cases. The experiment was carefully repeated with large numbers of grains, great care being taken to obtain thoroughly crushed material for the tests. After this was scattered, any grains not broken in the attrition process were removed with a needle before the grains whose tubes were to be measured were placed on culture. The results of the tests when these precautions were observed are given in table 4.

TABLE 4. *Summary of Results of Experiments to Determine if Pollen Tubes Give Greater Growth in the Presence of Crushed Pollen when Special Precautions are Taken to Secure Thorough Breaking up of the Grains (Lengths in Microns)*

Test	Without Crushed Grains		With Crushed Grains		Difference
	No. of Tubes	Average Length	No. of Tubes	Average Length	
1	120	791	156	789	- 2
2	110	579	108	481	+98
Average	230	690	264	663	-27

It is evident that, when the concentration of pollen killed by crushing is increased on the culture, greater growth does not result. The substance or substances promoting increased growth, whatever their nature may be, are apparently products of the metabolism of the pollen tube; they are readily diffusible and are utilized more completely when the tubes are massed.

The point having been established that the grouping of grains conduces to a greater amount of growth, the question was asked whether this was due to an acceleration of the rate from the start or to a prolongation of the growth period. A knowledge of their effect upon rate of growth might afford a clue to the nature of the substances at work. Accordingly, an experiment was arranged to determine the respective rates of growth of pollen tubes growing singly and when grouped with others. Each group consisted of nine grains, among which the single one whose behavior was to be followed was introduced after the others had attained a length of about 200  $\mu$ . Since the growth-promoting substances appeared to be products of metabolism, it was thought that this procedure would insure their presence in an effective

concentration from the start. The series of isolated grains was arranged on the same agar plate some distance from the groups. In table 5 the average times required for the tubes in the two series to reach lengths of  $410\ \mu$  and  $710\ \mu$  respectively are given.

TABLE 5. *Rates of Growth of Cucumis Pollen Tubes Grown Singly and in Groups*

	No. of Tubes	Time in Minutes Required to Reach Length of $410\ \mu$	No. of Tubes	Time in Minutes Required to Reach Length of $710\ \mu$
Singles . . .	10	45	6	76
Groups . . .	10	46	6	74

As shown in table 5, the rates of growth of isolated tubes and of tubes from groups of *Cucumis* pollen grains are not significantly different up to the stage when each series reaches a length of  $710\ \mu$ . The experiment was repeated with pollen from the same form, with essentially similar results. It is beyond this stage that the difference in rate of growth becomes evident; the growth of the isolated tubes soon slows down and finally ceases, while the tubes from the groups continue to elongate steadily until eventually their growth also subsides. It should be pointed out in connection with this test that *Cucumis* pollen tubes grow very rapidly; that they were grown on a watery medium and that the excess of water, influencing the growth rate to a considerable degree as it must, probably obscures any accelerating effect of the substances in question. With a form in which the pollen tubes grew more slowly than those of *Cucumis* on artificial media, it was thought that any acceleration might be more easily detected. Pollen of *Vinca minor* fulfilled this condition and was used in a single experiment arranged as before. The results are given in table 6.

TABLE 6. *Rates of Growth of Vinca Pollen Tubes Grown Singly and in Groups*

	No. of Tubes	Average Time in Minutes Required to Reach Length of $200\ \mu$	No. of Tubes	Average Time in Minutes Required to Reach Length of $200\ \mu$
Singles . . .	8	54	3	140
Groups . . .	12	40	8	68

Germination was rather poor in this experiment, and growth was irregular. Made at the end of the season, want of further pollen unfortunately precluded its repetition. The differences in rate of growth incident upon grouping appear to be very striking, but, as is shown, the values are based on small numbers of tubes. If further experiments bear out these results, we might conclude with considerable justification that the growth-promoting substances whose activities are revealed by grouping are catalytic in nature.



As shown by the studies of Jost (1907), Martin (1913), and East and Park (1918), self-sterility results from the failure of the pollen tubes after an incompatible pollination to grow fast enough in the style to reach the ovary and effect fertilization during the life of the flower. The cause of sterility is to be sought among the factors affecting the rate of growth. Various theories have been advanced to account for this condition. Jost (1907), in seeking an interpretation of the phenomenon, has postulated "Individuellestoffe," specific substances in self-sterile plants which inhibit pollen-tube growth. The sterility relations prevailing among the heterostyled forms, Jost believes, are best interpreted by assuming quantitative differences in the "Individuellestoffe." Correns (1912) advanced a somewhat similar hypothesis. East and Park (1918) have interpreted the facts in a different way. These authors state that:

Pollen tubes in a selfed pistil are not inhibited in their growth by substances secreted in that pistil, but rather that a substance or substances are secreted in the pistil after a compatible cross which accelerate growth, and that the direct cause of this secretion is a catalyser which the pollen-tube nucleus is able to produce because the zygotic constitution of the plant producing it is different in certain particular hereditary factors from that of the plant on which it is placed.

On this hypothesis, there is some local reaction between the pollen tube and the adjoining cells whereby the nutrients necessary for tube growth are made available. It is pointed out that the effect must be purely local, since the presence of compatible pollen tubes does not accelerate the growth of incompatible ones.

Put in its simplest form, an adequate interpretation of the facts regarding pollen-tube behavior in self-sterile forms must explain why, in the self-sterile but cross-fertile plants *A* and *B*, the two individual pollens react differently to the stylar environment of each plant. If the growth-promoting substances diffusing from pollen tubes, as demonstrated in artificial cultures, play the essential rôle in determining fertility or sterility (on the plant), they should exhibit the specificity that these relations demand; tubes from the pollen of *A* and *B* respectively should produce substances that are qualitatively different. Are we dealing here with a single substance produced in common by all pollen tubes, or with a class of substances alike only within compatible groups? It is upon the answer to this question that we must rest our judgment regarding their significance in self-sterility.

In investigating this matter, the most suitable material perhaps would be pollen easily cultured *in vitro* and from a self-sterile species in which the self-sterility relations had been determined. Pollen satisfying both these conditions, however, was not available, and the experiments were made with pollen fulfilling the former requirement in a satisfactory way and taken from two widely different species, namely, *Cucumis sativus*, a cucurbit, and *Vinca minor*, belonging to the Apocynaceae. If the growth-promoting substances produced by pollen tubes are specific in their nature, the growth of neither should be stimulated in the presence of the other.



Preliminary tests were made in order to determine approximately the size of group required in the case of *Vinca* pollen to give a decided increase in growth. The results as summarized in table 7 show that clusters of four grains give a markedly greater growth than isolated grains.

TABLE 7. *Growth of Vinca minor Pollen Tubes Grown Singly and in Quartets (Lengths in Microns)*

Test	Singles		Quartets		Difference
	No. of Tubes	Average Length	No. of Tubes	Average Length	
1	79	398	86	654	255
2	87	359	93	767	408
Average	166	378	179	712	355

The difference, amounting to 88.8 percent, is abnormally high perhaps, but the point is clearly established that the large, vigorous tubes of *Vinca* freely produce some substance or substances which stimulate their own growth at least. Will these substances stimulate the growth of the pollen tubes of *Cucumis*?

TABLE 8. *Growth of Cucumis Pollen Tubes when Grown Singly, in Quartets, and in Groups of Vinca Pollen Tubes on Sugar-agar-yeast Media (Lengths in Microns)*

Test	Singles		Quartets		Singles in Groups of <i>Vinca</i> Tubes	
	No. of Tubes	Average Length	No. of Tubes	Average Length	No. of Tubes	Average Length
1	124	459			51	522
2	112	453	123	437	61	685
3	81	1142	103	1134	26	2032
Average	317	631	226	755	138	879

To answer this question, seven carefully repeated tests were made, three on a 2-percent agar medium containing ten percent cane sugar and a little sterile yeast, and four tests on a medium lacking the latter constituent. In each test except one, three series of *Cucumis* pollen grains were arranged on a single agar plate, the first series consisting of isolated grains, the second of clusters of four; in the third series, groups of four *Vinca* pollen grains were arranged in succession, and after these had germinated a single *Cucumis* pollen grain was inserted in each group. When elongation had ceased, the average length of the *Cucumis* pollen tubes was determined for each series. The results of the tests on the yeast and yeast-free media are given in tables 8 and 9 respectively.

TABLE 9. *Growth of Cucumis Pollen Tubes when Grown Singly, in Quartets, and in Groups of Vinca Pollen Tubes on Sugar-agar Media (Lengths in Microns)*

Test	Singles		Quartets		Singles in Groups of Vinca Tubes	
	No. of Tubes	Average Length	No. of Tubes	Average Length	No. of Tubes	Average Length
1	104	384	133	453	58	394
2	79	379	97	628	49	1040
3	150	241	108	328	53	335
4	112	443	108	796	69	826
Average	445	350	446	544	229	649

It will be noted in table 8 that in tests 2 and 3 the average growth of *Cucumis* pollen tubes in quartets is slightly less than in the series from isolated grains. The difference is very slight, however, and, when the first test which did not involve a series of grouped *Cucumis* grains is considered, the average of the tests shows a balance in favor of the quartets of 19.6 percent, a value which more nearly approximates the difference we should expect in view of the results of several other similar tests previously made. In all the other tests the *Cucumis* groups gave a greater growth than the isolated grains; moreover, in all the tests but one the single *Cucumis* grains growing in groups of *Vinca* tubes gave greater average growth than either of the other two series. The differences in the amount of growth of *Cucumis* pollen under the three different conditions is clearly illustrated in table 10, where the amount of growth of the isolated grains on the two kinds of media is taken as 100 in each case, that of the other two series being given in proportion.

TABLE 10. *Comparative Amounts of Growth of Cucumis Pollen Tubes when the Grains are Isolated, in Quartets, and Inserted Singly in Groups of Vinca Pollen, the Amount of the First being Taken as 100*

Medium	Singles	Quartets	Vinca Groups
Sugar-agar-yeast.....	100	119.6	139.3
Sugar-agar.....	100	155.4	185.4

The increase in growth of *Cucumis* pollen tubes in the vicinity of *Vinca* tubes is very striking. Differences of 39.3 percent and 85.4 percent on yeast and yeast-free media respectively are undoubtedly significant. Contrary to expectation if the growth-promoting substances are specific in their action, it has been found that those produced by one species stimulate the growth of tubes of a distantly related one. The excess of growth of *Cucumis* tubes in the *Vinca* groups over the quartets of *Cucumis* pollen is possibly due to the comparatively greater growth of the *Vinca* tubes, with a consequent increase in the production of growth-promoting substances.

As East and Park (1918) have pointed out, the reaction between pollen tube and style must be mutual, otherwise the behavior of self-sterile plants between themselves could not be accounted for. It has been found that substances are produced during the growth of pollen tubes on artificial media that increase the amount and possibly the rate of growth. The tests we have made, however, do not indicate that these particular substances possess the degree of specificity that an explanation of self-sterility on the basis of their activity would demand.

#### THE FORMATION AND FUNCTION OF CALLOSE PLUGS

In the pollen tubes of *Gloxinia*, Strasburger (1878) first observed that the cavity of the much-elongated vegetative cell is frequently interrupted by short, hyaline structures which he termed *Propfen*. One year later, Elfving (1879) noted the same feature in pollen tubes growing *in vitro*. Numerous subsequent observations indicate their general occurrence. While it was first thought that these plugs are composed of cellulose, Mangin (1890) found that they consist of a closely related substance, callose. Bobilioff-Preisner (1917) reports that in *Vinca minor* the callose plugs may begin to form when the pollen tube is about 1 mm. long and continue to develop at more or less regular intervals of 300  $\mu$  as elongation proceeds. When a plug has completely occluded the cavity of the tube, it was observed that other plugs still continue to form in the portion cut off from the tip.

In the comparatively large pollen tubes of *Vinca minor*, the formation of the plugs can be readily observed. In our experiments the pollen was cultured on agar media containing 10 percent cane sugar and a little sterile yeast. At 28° C., germination takes place within 45 minutes. After about 5 hours, plugs may be seen in a few of the longer tubes. As Bobilioff-Preisner (1917) has noted, the plugs arise from around the wall of the tube (figs. 13, 14).<sup>2</sup> As the ring of callose thickens, the passage becomes gradually narrower until, as is shown in figure 15, it is completely closed. Further deposition of callose results in the longitudinal extension of the plug (fig. 16). Bobilioff-Preisner (1917) describes other methods of plug-formation in *Narcissus angustifolius* and *Lathyrus latifolius*, but the end result is the same, the distal portion of the tube becoming shut off from the proximal end. While the first plugs are laid down at more or less regular intervals, our observations agree with those of Bobilioff-Preisner that in the section of the tube cut off from the tip plugs continue to form at random and eventually fill a considerable portion of the cavity. Plug-formation continues throughout the growth period and for some time after the tube has reached its maximum length. In a *Vinca* tube 4 mm. long, as many as

<sup>2</sup> The figures referred to in this and the following paragraphs (figs. 13-23) were published in Plate XX, accompanying the third article of this series (in the issue of this JOURNAL for June, 1924).

14 completely formed plugs have been counted; and between some of these, considerable additional callose had been deposited around the walls. The first plug formed usually lies from  $400\ \mu$  to  $700\ \mu$  from the pollen grain; at the other end of the tube no plugs have been observed within  $200\ \mu$  of the tip. After a plug has formed, no further elongation takes place between it and the pollen grain. One tube, kept under observation after the passage had been closed, grew  $600\ \mu$  during the 3 hours following without altering the distance between the plug and the grain.

Following germination, the food materials stored in the pollen pass into the growing tube (figs. 17, 18). In *Vinca minor* these substances are fats, and their distribution can be readily followed by staining with Sudan III. As the tube elongates, this fatty material is carried forward in the region of the tip (fig. 19), leaving the cytoplasm in the body of the tube clear and translucent as shown in figure 13. It is in this latter region that the callose plugs form. Unfortunately, in *Vinca* the distribution of the nuclei could not be easily followed. Only two tubes were seen in which they could be distinguished with certainty. One is represented in figure 23 with a single nucleus at the tip; in the other, two nuclei were present and similarly situated. It is a reasonable supposition that in the pollen tubes of *Vinca minor* growing *in vitro* the nuclei lie imbedded in the matrix of fat globules toward the tip.

When a plug is formed, the tip of the pollen tube in which its activity is centered becomes shut off from the parent grain and from the older portion of the tube where elongation has ceased. As growth proceeds, successive plugs are laid down and the size of the active vegetative cell is maintained within fairly constant limits. That the tip of the tube shut off from the parent grain by a callose plug is capable of independent existence was demonstrated by the following experiment: A culture of *Vinca* pollen about 5 hours old in which plugs were forming freely was placed on the stage of a dissecting microscope. Three tubes were selected, each showing a well formed plug. Each of these pollen tubes was cut in two with fine curved scissors just at the callose plug on the side remote from the tip, and the grain end was discarded. The distance between the plug and the tip on the other piece was measured, and the culture was set away for  $1\frac{1}{2}$  hours. At the end of this time the lengths of the tubes were again determined. In two cases, no elongation had taken place; in the third, the isolated tip end of the tube had grown  $469\ \mu$ . In the first two cases, growth had very probably ceased before the operation was made. To check the matter, a single tube found to be actively growing was similarly treated and the portion between the callose plug and the tip was kept under observation. In the three hours following the removal of the older portion of the tube, the remaining part, closed at the proximal end by the plug, grew  $734\ \mu$ . If a tube is cut in two in which no plug has been formed or in which the cavity has not become completely closed, the protoplasmic contents ooze out at the severed end and growth ceases at once.

The curve of growth obtained by plotting length of tube against time for *Vinca* growing on artificial media is somewhat S-shaped (text fig. 2 of the third paper of this series). The first plug is usually formed about the time of most rapid elongation and causes no noticeable change in the shape of the growth curve. During the later stages, as growth slows down and after elongation has ceased entirely, plugs form with greater frequency. Since plug-formation does not alter the rate of growth and often occurs in non-nucleated sections of the tube which, moreover, are quite free from reserve food material, it would appear that this deposition of callose is a secondary phenomenon which accompanies waning activity. Benson (1894) found that the pollen tubes of *Carpinus* growing in an artificial medium often became plugged just at the junction with the grain and sometimes immediately above the enlarged tip. It is stated that the tip of the tube thus becomes virtually "an isolated spore like a pollen grain, which, though it had lost its outer coat, would in the normal state of things be safely housed or encysted in the tissue of the style." In many woody plants, as described by Coulter and Chamberlain (1915), weeks or even months may elapse between pollination and fertilization. It appears that in these cases the pollen tubes grow into the style and become dormant for a time, resuming growth at a later period. It may well be that in these forms, as Benson (1894) has suggested in the case of *Carpinus*, callose plugs, forming near the tips as growth declines, serve to isolate them during their quiescent period is a more or less spore-like condition.

Miller (1919) has reported that pollen tubes in maize do not at any time extend the whole length of the silk. Just how general is this dissolution of the older parts of the pollen tube is uncertain. In preparing material for cytological examination by the ordinary methods, the reagents used may remove in large part such vestiges of the tube as remain behind the nucleated tip. In the styles of *Nicotiana* and *Primula* killed with hot water, tubes may be traced through the tissues for a considerable distance. We may reasonably suppose, however, that in many long-styled forms the tubes are not continuous from stigma to ovary. From the results of our experiments, showing that the portion of the tube from a fully formed plug forward to the tip is capable of independent and continued growth, it is logical to conclude that in species where the tube does become separated from the grain the formation of callose plugs in series serves to maintain the integrity of the vegetative pollen cell. It should be noted also that by cutting off the older part of the tube the plug limits the region from which the vegetative cell absorbs nutrient materials to the less exhausted portions of the style.

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due the Department of Genetics of the University of Wisconsin for affording the writer an opportunity to complete the paper while a member of its staff.

#### SUMMARY AND CONCLUSIONS

1. A method is described that permits an accurate determination of the rate and amount of pollen-tube growth on artificial media.
2. A very considerable improvement in culture media for pollen was effected through the discovery that sterile yeast added in small amounts to sugar media stimulates growth. In four tests, the pollen tubes of *Cucumis* showed an average percentage increase in length of 60 percent on the addition of yeast; in six tests with *Primula* pollen, the growth was similarly increased 72 percent; the addition of yeast to the media in six tests with *Lythrum* pollen increased the average length of the tubes 142 percent.
3. The growth-promoting substance or substances in the sterile yeast increase the amount but do not alter the type of growth. They are water-soluble, heat-stable, and active in small amounts. It is suggested that they may be protein derivatives or possibly of a vitaminic nature.
4. The addition of raw potato juice and extracts of gynoecium parts to artificial media regularly increases the growth of pollen tubes.
5. No evidence was secured to show that nitrogen in the form of inorganic salts promotes growth. Glycocoll and asparagin gave negative results. There was some indication, however, that peptone stimulates growth.
6. It is concluded that pollen tubes may utilize cane sugar, and very probably glucose and fructose, as sources of carbon.
7. The bursting of tubes so frequently encountered in the artificial culture of pollen is interpreted as an osmotic phenomenon.
8. Pollen-tube growth is markedly depressed in the presence of small amounts of various inorganic salts or when sea water is added to the culture medium in concentrations as low as 12 percent. Future studies on the cultural requirements of pollen must be made under laboratory conditions that will permit of an exact control of minute amounts of the various constituents entering into the media.
9. With pollen from *Vinca*, *Scilla*, *Chionodoxa*, *Puschkinia*, and *Muscari*, tubes were secured on artificial media as long as, or longer than, the styles of the respective plants.
10. The growth curve of pollen tubes cultured *in vitro* resembles that of an autocatalytic reaction where the amount of substrate is limited.
11. The digestion of the reserve material contributed to the growing tube by the pollen grain was observed and figured. The view is put forward that the form of the curve describing pollen-tube growth *in vitro* is dependent in large part upon the course of the autocatalytic reaction involved in the digestion of these reserve food substances.



12. The difference in form of the growth curves of pollen tubes growing *in vitro* and *in vivo* is best interpreted, we believe, as a result of the difference in the water relations in the two cases. In consequence of the excess of water in artificial media, the amount of synthesis of food material within the tube is almost negligible and the reaction goes almost entirely to the right; in the plant, on the other hand, the amount of water in the system is low and the hydrolytic and synthetic processes proceed at more nearly equal rates, the reserve materials in the tube are replaced from sugars derived from the style, and throughout the cycle of growth no deficiency of substrate is manifested.

13. Anatomical features of the style appear to be sufficient to account for the direction of pollen-tube growth therein. Chemotropism has been demonstrated in the pollen tubes of comparatively few forms. Its importance in determining the direction of pollen-tube growth in the ovarian cavity is problematical.

14. Increases in growth amounting to 34.6 percent and 44.5 percent were obtained on culturing pollen in groups of 4 grains each on yeast and yeast-free media respectively. This increased growth is interpreted as the result of the more complete utilization by the groups, through the proximity of their members, of some diffusible growth-promoting substance or substances, of a catalytic nature perhaps, that are produced by the growing tube.

15. Our experiments have not shown that these particular growth-promoting substances possess the degree of specificity that an explanation of the phenomenon of self-sterility on the basis of their action would demand.

16. It was demonstrated experimentally that the tips of pollen tubes cut off from the older portions by callose plugs are capable of independent growth. Observations were made on the formation of these plugs, and their function in pollen-tube growth is discussed.

17. The distribution of the nuclei in pollen tubes grown artificially was observed in detail and figured. The facts regarding migration and the division of the generative nucleus to form the sperm nuclei indicate close agreement in the behavior of the nuclei in pollen tubes growing *in vitro* and *in vivo*.

18. The literature on the physiology of pollen has been reviewed *in extenso*.

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# THE RELATION OF SOIL TEMPERATURE TO GERMINATION OF CERTAIN PHILIPPINE UPLAND AND LOW- LAND VARIETIES OF RICE AND INFECTION BY THE HELMINTHOSPORIUM DISEASE

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## INTRODUCTION

The Helminthosporium disease of rice (*Oryza sativa* L.) caused by *Helminthosporium oryzae* Breda de Haan is of serious economic importance on rice seedlings in the seed beds or in the field (9). If weather conditions are favorable, this organism causes a serious seedling blight of rice in both the seed beds and the field. The greater part of the seedling infection by *H. oryzae* is due to seed-borne mycelium, although some infection may be due to mycelium and conidia overwintering in the soil or in crop residue. Nishikado and Miyake (7, 8) have shown that the infection from seed-borne mycelium may be reduced considerably by treating air-dry kernels with water at 54° to 55° C. for 10 to 15 minutes, or by treating rice kernels, which have been soaked in water at room temperature for 24 hours, at 53° C. for 10 minutes, or at 54° C. for five minutes. Very little work has been done upon the relation of soil temperature to the germination of rice. Nagai (6) reports that 91 to 94 percent of "well dried" rice grains germinate after exposure to heat at 97° to 98° C. for two hours, whereas "air-dried" grains, heated to these temperatures for the same length of time, do not germinate. Nagai does not, however, describe the method used in obtaining well dried grains. Fanno (1), studying the influence of temperature and moisture on five varieties of rice and three varieties of corn, found that treatment with dry heat at 90° C. for one hour was fatal to both rice and corn.

As rice is a tropical or subtropical crop, experiments were conducted with the hope that the difference in temperature relations of the rice seedlings and of the seedling blight fungus might be such as to permit of some application of results to disease control. The growth of *H. oryzae* in pure

<sup>1</sup> The work reported in this paper was conducted in the Department of Plant Pathology of the University of Wisconsin. The writer acknowledges his indebtedness to Professor L. R. Jones, under whose general advice this work was undertaken. Thanks are due to Dr. E. J. Kraus for suggestions on soil-temperature experiments, to Dr. James G. Dickson for suggestions and assistance on soil-temperature experiments and in the preparation of the manuscript, and to Dr. James Johnson for the use of his constant-temperature and humidity chambers.

culture at different temperatures has already been noted, and a discussion of this is given in an earlier paper (11). During the last two years the writer has conducted experiments at Madison, Wisconsin, to determine the soil temperatures best suited to the germination of certain Philippine varieties of upland and lowland rice. At the same time experiments were conducted to determine the influence of soil temperature on the infection of seedlings by *H. oryzae*.

Haberlandt (2), working with warm-climate plants in heated chambers, found that the minimum temperatures for sorghum, rice, and other plants are 10° to 12° C., 10° to 12° C., and 12° to 15° C. respectively. In chambers heated from 10° to 45° C. at 5° C. intervals he found that the minimum temperature for germination of rice is 10° to 12° C., the optimum 30° to 32° C., and the maximum 36° to 38° C. From the results of his experiments Haberlandt concludes that the minimum temperatures oscillate more than the maximum. The minimum varies between 1° and 14° C., the maximum, with few exceptions, between 35° and 40° C. The difference between the minimum and maximum temperatures for germination varies greatly for different varieties. The cool-temperature species have a wider range than the warm-temperature species. The writer found that the optimum temperature for the emergence of Philippine rices is 28° to 32° C., the minimum 16° C., and the maximum 36° to 40° C.

#### GERMINATION OF PHILIPPINE UPLAND AND LOWLAND VARIETIES OF RICE AT DIFFERENT SOIL TEMPERATURES

The experiments the results of which are discussed in this paper were carried out in the temperature tanks of the Department of Plant Pathology of the University of Wisconsin. The history of the Wisconsin soil-temperature tanks, a description of them, and the methods of operation are given by Jones (4, 5). The different varieties of rice used in this work were obtained from the Department of Agronomy of the University of the Philippines at Los Baños, Laguna, Philippine Islands. A silt-loam soil containing a high percentage of organic matter was obtained from a wood lot which had been uncropped for at least 20 years. This soil was used in all the experiments with the exception of the last four series conducted during the winter of 1922-23. The soil was carefully sifted through wire sieves. The sifted soil was autoclaved for one to two hours at 15- to 20-pounds' pressure. The cans were washed and autoclaved in order completely to sterilize them. The weights of the cans were made the same by the addition of clean sterilized pebbles. The sterilized soil was thoroughly mixed, after which an equal known weight of soil was placed in each can. The moisture, based on the dry weight of the soil, was determined as well as the water-holding capacity of the soil. The water-holding capacity of the soil was used as a basis for regulating moisture, rather than

the dry weight, as the moisture available to roots of plants varies with the kind of soil as well as with its texture and composition. The soil taken at different times contained different amounts of moisture and had different water-holding capacities. The kernels of rice were planted in furrows one centimeter deep except in the last four series of experiments on the type of germination of rice at different soil temperatures, in which rice was planted three centimeters deep. The kernels were set sufficiently far apart to prevent them from touching each other or the sides of the cans. The tops of the cans were covered with loose-fitting glass covers until after emergence. The water lost by evaporation and transpiration was returned to the soil by bringing the cans to their original weights at regular intervals. In the first experiments, the temperatures used were from 20° to 44° C. at 4° C. intervals. In succeeding experiments, temperatures from 12° to 40° C. at 4° C. intervals were used. Since the Philippine varieties of rice did not germinate at 12° C. and only poorly at 40° C., these two temperatures were not used in the last experiments.

The preliminary experiments performed during May, June, and July, 1921, are not reported here. Only succeeding experiments beginning August, 1921, are discussed. In August, 1921, a parallel series of moisture experiments were conducted in the temperature tanks. The soil used contained 16 percent moisture based on its dry weight, and had a water-holding capacity of 84.6 percent. The soil moisture of one half of the series was made up to 16 percent of the water-holding capacity. The soil moisture of the other half of the series was brought up to 100 percent of the water-holding capacity, *i.e.*, the soil was saturated with water. The varieties 7369 F4 Macan, 7328 F2 Roxas, 10231 F2 Binangbang, and 6040 F6 Kinagaykay were planted, 50 kernels per pot, on August 8, 1921.

In the next experiment, the same kind of soil to which a small amount of sand had been added was used. The soil had 21.5 percent moisture based on its dry weight, and a water-holding capacity of 14.8 percent. The moisture of one of the series was brought up to 68.4 percent of the water-holding capacity, and the other to 100 percent. Fifty kernels each of 7328 F2 Roxas and 6040 F6 Kinagaykay were planted in each can on September 2, 1921.

In December, 1921, the third experiment was started, using sterilized soil which was taken from the woodlot. The soil had 17 percent moisture based on the dry weight, and a water-holding capacity of 16.6 percent. Twenty kernels of the upland rice 5891 F6 Binicol and 20 of the lowland rice 7328 F2 Roxas were planted on December 24, 1921. After planting, the moisture of the soil was brought up to 20 percent of the water-holding capacity.

The fourth experiment was performed during the winter of 1921-22, using the remainder of the soil having the same properties as that in the third experiment. In this test, 25 grains of the upland variety 9118 Tini-



aong were planted on January 24, 1922, and the soil moisture was made 20 percent of the water-holding capacity. The results of the four series of germination experiments of rice at different soil temperatures are given in table I. The relative sizes of the seedlings at different soil temperatures,

TABLE I. *The Influence of Soil Temperature on the Emergence of Philippine Varieties of Rice*

Soil Temp. ° C.	Soil Moisture Based on Water-holding Capacity	Number of Days to Emerge	Percentage Emergence					
			7369 F4 Macan	7328 F2 Roxas	10231 F2 Binang-bang	6040 F6 Kinagay-kay	5891 F6 Binicol	9118 Tiniaong
24	16	5	100	100	100	100	—	—
28	16	3	100	100	100	100	—	—
32	16	2	100	100	100	100	—	—
36	16	2	100	100	100	100	—	—
40	16	2	80	84	86	83	—	—
24	100	12	98	100	100	100	—	—
28	100	8	98	95	98	100	—	—
32	100	3	98	100	100	98	—	—
36	100	3	86	88	100	96	—	—
40	100	0	0	0	0	0	—	—
20	68.4	8	—	96	—	94	—	—
24	68.4	5	—	96	—	98	—	—
28	68.4	3	—	94	—	98	—	—
32	68.4	2	—	94	—	96	—	—
36	68.4	2	—	94	—	98	—	—
40	68.4	5	—	82	—	86	—	—
20	100	13	—	94	—	96	—	—
24	100	7	—	96	—	98	—	—
28	100	6	—	94	—	98	—	—
32	100	4	—	96	—	98	—	—
36	100	4	—	96	—	96	—	—
40	100	6	—	80	—	84	—	—
20	20	9	—	100	—	—	100	—
24	20	6	—	100	—	—	100	—
28	20	4	—	100	—	—	100	—
32	20	3	—	100	—	—	100	—
36	20	3	—	100	—	—	100	—
40	20	4	—	60	—	—	40	—
20	20	9	—	—	—	—	—	92
24	20	5	—	—	—	—	—	92
28	20	3	—	—	—	—	—	96
32	20	3	—	—	—	—	—	92
36	20	2	—	—	—	—	—	80
40	20	4	—	—	—	—	—	20

10 days after planting, are shown in Plate XXIX. The low percentage of emergence of the variety 9118 Tiniaong used in the fourth experiment may be attributed to the poor quality of the seed, which was not secured from carefully selected plants or from plants grown under controlled conditions.



Table 2 shows in summary the influence of soil temperature and moisture on the rate and percentage of emergence of the seedlings of Philippine rices, the average number of days required for emergence from soils with moisture varying from 16 to 100 percent of the water-holding capacity and at temperatures from 20° to 40° C., and the average percentage of emergence.

TABLE 2. *The Influence of Soil Temperature and Moisture on Rate and Percentage of Emergence of Philippine Rices (Summary of Table 1)*

Soil Temp. ° C.	Soil Moisture Based on Water-holding Capacity							
	16 percent		20 percent		68.4 percent		100 percent	
	Average Number of Days to Emerge	Average Percentage Emergence	Average Number of Days to Emerge	Average Percentage Emergence	Average Number of Days to Emerge	Average Percentage Emergence	Average Number of Days to Emerge	Average Percentage Emergence
20	..	..	9.0	96	8.0	95	13.0	95.0
24	5.0	100	5.5	96	5.0	97	9.5	98.6
28	3.0	100	3.5	98	3.0	96	7.0	97.1
32	2.0	100	3.0	96	2.0	95	3.5	98.3
36	2.0	100	2.5	90	2.0	96	3.5	93.6
40	2.0	83.2	4.0	40	5.0	84	6.0	82.0

The results as summarized in table 2 show that Philippine rices emerged in from 2.0 to 2.5 days at 36° C., 2.0 to 3.0 days at 32° C., 2.0 to 5.0 days at 40° C., 3.0 to 3.5 days at 28° C., 5.0 to 5.5 days at 24° C., and 8.0 to 9.0 days at 20° C., when the soil moisture was from 16 to 68.4 percent of the water-holding capacity of the soil. The figures further show that, when the soil moisture was 100 percent of the water-holding capacity, emergence at 20° C. was delayed 4.0 to 5.0 days, at 24° C. 4.0 to 4.5 days, at 28° C. 3.5 to 4.0 days, at 32° C. 0.5 to 1.5 days, at 36° C. 1.0 to 1.5 days, and at 40° C. 1.0 to 4.0 days.

In all the previous experiments conducted, the soil used was autoclaved for one to two hours at 15- to 20-pounds' pressure. In order to determine whether sterilization with heat under high pressure for a long period produces toxic substances in the soil which might inhibit the germination of rice, an experiment was started on June 9, 1922, to compare the rate and percentage of emergence in sterilized and unsterilized soil at different soil temperatures. In one half of the series the soil taken from the wood lot was autoclaved, and in the other half the fresh, unsterilized soil was used. The moisture content based on dry weight of the series with unsterilized soil was 22 percent, and the water-holding capacity was 34 percent. The moisture content of the sterilized soil was also 22 percent, but the water-holding capacity after sterilization was increased to 50.8 percent.

Four varieties of upland rice—9118 Tiniaong, 9047 Kinandang kinapal, 4495 F5 Putyucanon, and 91112 Lubuang, and four of lowland rice—

17565 Daluson, 17566 Kinastila, 7365 Inasimang, and 17567 Murmuray, were planted, ten kernels per can, in furrows one centimeter deep. The percentage emergence and the rate of emergence of the seedlings showed that in both sterilized and unsterilized soil the different varieties of upland and lowland rice germinated and emerged at the same time at any given temperature. Emergence at 36° C. took place 2 days after planting; at 32° and 28° C., 3 days; at 24° C., 4 days; at 20° C., 5 days; and at 16° C., 10 days, after planting. The emergence percentages were higher at 36°, 32°, and 28° C. in the sterilized soil than in the unsterilized soil, perhaps because of the control of other soil fungi. Germination was poor at 16° and at 40° C. in both sterilized and unsterilized soil. At 24° and 20° C., the rate and percentage of emergence in both series were the same.

The previous experiment was duplicated on August 3, 1922, using the remainder of the soil that was left from the other experiment. The results obtained were identical with those in the first experiment. As rice planted in both sterilized and unsterilized soil emerged at the same time in any given temperature, the writer believes that there were no visible effects of soil-sterilization upon the germination of rice. The effect on the subsequent growth of the plants was not determined in these experiments.

The variations in the percentages of emergence of rice in both experiments do not seem to be due to the fact that one of the soil lots was sterilized and the other was not, nor do they seem to be due to the difference in the amount of moisture in the soil. The writer believes that these variations were due chiefly to the quality of the seed and to the influence of soil temperatures on the germination processes and upon fungus invasion. When these varieties of rice were tested for germination, it was found that none of them was entirely free from seed-borne organisms, *Helminthosporium oryzae* and other fungi, the identity of which the writer did not determine. In an experiment of this sort, seed of known history, produced under controlled conditions, should be used. However, in these experiments it was not possible to obtain such seed, so rice obtained from the Philippines was used. Moreover, it was not possible to select kernels free from seed-borne fungi. The results show, however, that at from 28° to 36° C. there were higher and more uniform percentages of germination. The figures seem to show that below 28° C. the organisms within the grains had a better chance to penetrate the slowly emerging seedlings than at higher temperatures at which the seedlings emerged more quickly. On account of infection of seedlings by soil fungi and the seed-borne *H. oryzae*, there was a less uniform stand of the seedlings at 16° to 24° C. Furthermore, at 16° to 24° C. the percentages of germination were uneven. It was further noted that the seedlings at lower soil temperatures were more succulent and crisp than those at higher temperatures. This condition probably favored the infection and invasion by either *H. oryzae* or other fungi.

THE INFECTION OF CERTAIN VARIETIES OF PHILIPPINE RICE BY *HELMINTHOSPORIUM ORYZAE* AT DIFFERENT SOIL TEMPERATURES

The optimum temperature for the growth of *H. oryzae* in pure culture is from 24° to 32° C. The production of conidia by the sporulating strains takes place from 16° to 28° C. From 32° to 36° C. there is a tendency for the fungus to produce aërial mycelium (11). The cardinal temperatures for germination of rice have been given in connection with previous experiments. The six preliminary experiments on infection of rice during germination at different soil temperatures, which were conducted during the summer and fall of 1921, using temperatures from 20° to 40° C., are not discussed in detail in this paper. The inoculations were made either by placing portions of the media about the size of the caryopses, containing the fungous mycelium, beside the grains at the time of planting, or by rolling the moistened rice grains on the surface of 15- to 30-day-old potato-dextrose-agar cultures of the Louisiana and the Philippine strains of *H. oryzae* grown in petri dishes. The varieties of rice used were 7369 F4 Macan, 7328 F2 Roxas, 9118 Tiniaong, 10231 F2 Binangbang, 6040 F6 Kinagaykay, and 5891 F6 Binicol. Two soil moistures, one at 16 and the other at 100 percent of the water-holding capacity were used. The soil used was taken from the wood lot described previously, sifted, and sterilized for two hours at 20 pounds' pressure. The results show that infection of rice seedlings by this organism occurred at soil temperatures from 20° to 36° C. The development of the lesions on the seedlings was more rapid at temperatures from 28° to 32° C. A larger number of lesions and considerable blighting before emergence occurred at soil temperatures lower than 24° C.<sup>2</sup>

Three experiments were started in July, 1922, to determine the influence of soil temperature on seedling blight produced by the same organism. The moisture of the soil based on the dry weight was 22 percent, and the water-holding capacity was 27.5 percent. The same soil was used in all three experiments. After planting and inoculating, the soil moisture was brought up to 25 percent of the water-holding capacity. The seed for the three series was inoculated with the Japanese strain JNS1,<sup>3</sup> the Louisiana strain AIS,<sup>4</sup> and the Philippine strain PO<sup>5</sup> of *Helminthosporium oryzae* respectively (11). In each of the three experiments, 20 kernels of rice of the upland variety 9115 F2 Dinalaga were inoculated and planted on one

<sup>2</sup> In an abstract (Phytopath. 13: 53. 1923), it was erroneously stated that the disease is most severe at 28° to 32° C. The statement was intended to read that the development of the lesions on the seedlings is most rapid at 28° to 32° C., perhaps because of the rapid development of both rice and *Helminthosporium oryzae* at these temperatures.

<sup>3</sup> Strain JNS1 is culture 45 of Mr. Y. Nishikado, which was obtained from Dr. F. L. Stevens on September 14, 1921.

<sup>4</sup> Strain AIS is the sporulating strain of the Louisiana *H. oryzae*, developed on the cotton at the bottom of sorghum panicles July 26, 1921.

<sup>5</sup> Strain PO is the non-sporulating culture of the Philippine strain, isolated from single spores.

side of the can, whereas 20 kernels of the same variety were inoculated with sterile potato-dextrose agar and planted on the other side of the can as a control. In inoculating the rice grains, the entire growth of six-day-old cultures of the Philippine strain and 14-day-old cultures of both the Louisiana and the Japanese strains of *H. oryzae* growing on potato-dextrose agar in petri dishes were chopped into small pieces and thoroughly mixed with the kernels. The kernels in the checks were mixed with the same amount of sterile potato-dextrose agar. The first experiment was started on July 8 and completed on July 17, 1922. The results are given in table 3.

TABLE 3. *The Influence of Soil Temperature on the Infection of Rice Seedlings by Helminthosporium oryzae*

Soil Temp. °C.	Number of Days to Emerge		Percentage Emergence						Percentage Infection					
	Inoculated	Check	Inoculated			Check			Inoculated			Check		
			JNS <sub>1</sub>	PO	AIS	JNS <sub>1</sub>	PO	AIS	JNS <sub>1</sub>	PO	AIS	JNS <sub>1</sub>	PO	AIS
16	9	9	100	100	100	100	100	100	40	50	60	0	0	0
20	6	6	100	100	100	100	100	100	100	80	100	0	0	0
24	5	5	100	100	100	100	100	100	30	90	100	0	0	0
28	4	4	100	100	100	100	100	100	90	100	100	0	0	0
32	3	3	100	100	100	100	100	100	50	100	100	0	0	0
36	3	3	100	100	100	100	100	100	60	60	60	0	0	0
40	6	6	90	90	90	90	90	90	0	0	0	0	0	0

The data presented in table 3 show that the variety 9115 F2 Dinalaga germinated 100 percent in both the check and the inoculated series at soil temperatures from 16° to 36° C. At 40° C., the control and inoculated series germinated 90 percent. Seedling infection took place at from 16° to 36° C. In the series inoculated with the Philippine and the Louisiana

TABLE 4. *The Influence of Soil Temperature on the Infection of Rice Seedlings by Helminthosporium oryzae*

Soil Temp. °C.	Number of Days to Emerge		Percentage Emergence						Percentage Infection					
	Inoculated	Check	Inoculated			Check			Inoculated			Check		
			PO	JNS <sub>1</sub>	AIS	PO	JNS <sub>1</sub>	AIS	PO	JNS <sub>1</sub>	AIS	PO	JNS <sub>1</sub>	AIS
16	12	12	40	20	20	100	90	100	90	100	100	0	0	0
20	7	7	50	70	30	100	90	100	100	100	100	0	0	0
24	5	5	100	100	40	100	90	100	100	100	100	0	0	0
28	4	4	70	90	80	100	100	100	100	100	100	0	0	0
32	4	4	100	100	100	100	100	100	70	30	90	0	0	0
36	3	3	100	100	100	100	100	100	90	60	80	0	0	0
40	6	6	70	90	80	80	86	80	0	0	0	0	0	0

strains, 100 percent of the plants showed a light infection at soil temperatures of 28° and 32° C., whereas 90 and 50 percent of the plants showed a light infection in the series inoculated with the Japanese culture. The seedlings blighted in the series inoculated with the Japanese and the Philippine strains of *H. oryzae* at soil temperatures from 16° to 24° C.

In the second experiment the potato-dextrose-agar cultures of the three strains of the fungus were seven days old. The series was started on July 19, and the final records were taken on August 1, 1922. The results are given in table 4.

Table 4 shows that the Philippine, the Louisiana, and the Japanese strains of *H. oryzae* produced seedling infections at soil temperatures of 16° to 36° C. The influence of soil temperature upon the type of infection of the seedlings is shown in table 5.

TABLE 5. *The Influence of Soil Temperature on the Type of Infection of Rice Seedlings by Helminthosporium oryzae*

Soil Temp. °C.	Percentage of seedlings showing different types of infection								
	Philippine Strain			Louisiana Strain			Japanese Strain		
	Coleoptile	Leaf Spot	Blighting	Coleoptile	Leaf Spot	Blighting	Coleoptile	Leaf Spot	Blighting
16			90			100			100
20	50		50	30		70	70		30
24	20	40	40	20	30	50	40	20	40
28	50	20	30	40	40	20	30	30	40
32	10	50	10	40	50		30		
36	90			80			60		

The data given in table 5 show that the blighting of the seedlings occurred from 16° to 32° C. with the Philippine strain of *H. oryzae*, and from 16° to 28° C. with the Louisiana and the Japanese strains. With the Philippine strain, the percentage of blighted seedlings decreased from 90 percent at 16° C. to 10 percent at 32° C. The percentage of blight infection by the Louisiana strain decreased from a total destruction at 16° C. to 20 percent at 28° C. The Japanese strain produced total blighting at 16° C., 30 percent at 20° C., and 40 percent at 24° and 28° C. respectively. The leaf-spot infection occurred from 24° to 28° C. with the Japanese strain. With the Philippine and the Louisiana strains, leaf spot occurred from 24° to 32° C. with increasing severity with the rise in temperature. At 28° C., the leaf spot produced by the Philippine strain was less than at 24° C. and 32° C. Coleoptile infection produced by the three strains of *H. oryzae* occurred from 20° to 36° C. There was a decrease in the percentage of coleoptile infection from 20° to 24° C. with the Philippine and the Louisiana strains, and an increase at 28° C. The percentage of plants with coleoptile infections with all strains of the fungus was high at 20° and 36° C., with a

decrease in severity and amount of infection at the intervening soil temperatures.

Partly because of the unfavorable influence of lower temperatures on the germination of rice, the infection was more severe at soil temperatures from 16° to 24° C., which resulted in higher percentages of blighted seedlings. Most of the blighting occurred before the seedlings emerged. The difference, therefore, in stand between the inoculated and the check cans, especially at the lower soil temperatures, was very marked, as shown in Plate XXX.

In the third experiment, 20-day-old potato-dextrose-agar cultures of the Philippine, the Louisiana, and the Japanese strains of *H. oryzae*, PO, AIS, and JNS<sub>1</sub> respectively, were thoroughly minced and used for inoculating the seed. The work was started on August 3 and completed on August 14, 1922. The results are given in table 6.

TABLE 6. *The Influence of Soil Temperature on the Infection of Germinating Rice by Helminthosporium oryzae*

Soil Temp. °C.	Number of Days to Emerge		Percentage Emergence						Percentage Infection					
			Inoculated			Check			Inoculated			Check		
	Inoculated	Check	PO	AIS	JNS <sub>1</sub>	PO	AIS	JNS <sub>1</sub>	PO	AIS	JNS <sub>1</sub>	PO	AIS	JNS <sub>1</sub>
16	Rice not sprouted													
20	7	7	70	70	100	80	90	90	100	100	100	0	0	0
24	4	4	100	90	100	100	100	100	80	100	80	0	0	0
28	2	2	90	100	100	100	100	100	90	100	100	0	0	0
32	2	2	90	100	100	100	100	100	10	30	20	0	0	0
36	1	1	100	100	100	100	100	100	100	70	50	0	0	0
40	Rice barely emerging. Emergence was not recorded													

The results presented in table 6 show that the Philippine, the Louisiana, and the Japanese strains of *H. oryzae* produced infection from 20° to 36° C. The types of infection produced by the three strains are given in table 7.

TABLE 7. *The Influence of Soil Temperature on Type of Seedling Infection by Helminthosporium oryzae*

Soil Temp. °C.	Percentage of Seedlings Showing Different Types of Infection											
	Philippine Strain, PO				Louisiana Strain, AIS				Japanese Strain, JNS <sub>1</sub>			
	Cole-optile	Leaf Spot	Blighting	Uninfected	Cole-optile	Leaf Spot	Blighting	Uninfected	Cole-optile	Leaf Spot	Blighting	Uninfected
16												
20	50	0	50	0	70	0	30	0	70	0	30	0
24	60	0	20	20	90	0	10	0	80	0	0	20
28	70	0	20	0	80	0	20	0	90	0	10	0
32	10	0	0	80	30	0	0	70	10	0	10	80
36	80	0	20	0	70	0	0	30	50	0	0	50



The results presented in table 7 show that rice seedlings were blighted by the Philippine strain of *H. oryzae* at soil temperatures from 20° to 36° C. The infection decreased from 50 percent at 20°C. to 20 percent at 24°C. The percentage of blight infection from 24° to 36° C. remained the same, except at 32° C., where no blighting occurred. The Louisiana strain of *H. oryzae* produced 30, 10, and 20 percent of blight infection at 20°, 24°, and 28° C. respectively. The Japanese strain produced 30 percent of blighted seedlings at 20° C., but no seedlings were blighted at 24° C. At 28° and 32° C., seedling blight was produced in 10 percent of the seedlings. In this experiment, no leaf spot was noted on seedlings from the kernels inoculated with any of the three strains. Infection of the coleoptile increased from 20° to 28° C. At 32° C., the coleoptile infection produced by the three strains of *H. oryzae* was less severe than at 28° and 36° C. At 36° C., the infection of the coleoptile by the Japanese and Louisiana strains was less than that at 28° C., whereas the Philippine strain produced a higher coleoptile infection at 36° than at 28° C.

THE TYPE OF GERMINATION OF RICE AT DIFFERENT SOIL TEMPERATURES  
AND ITS RELATION TO THE SEVERITY OF INFECTION BY  
*HELMINTHOSPORIUM ORYZAE*

During the latter part of the winter of 1922-23, four series of experiments were conducted in the soil-temperature tanks to determine the relation of type of germination of rice at different soil temperatures to the production and severity of infection by *Helminthosporium oryzae*. Sterilized, well mixed garden loam was used in these experiments. This soil contained 24 percent moisture based on the dry weight. The moisture-holding capacity of the soil was 45.25 percent. Each can was separated into two compartments by means of a glass plate driven into the soil. Each compartment was planted with 50 kernels of 5891 F6 Binicol rice. As the pathogenicity of the Japanese, the Louisiana, and the Philippine strains of *H. oryzae* had been proved to be about the same in the preceding experiments, only the Louisiana strain AIS was used for inoculating the kernels in this experiment. The 50 kernels on one side of the can were inoculated by chopping 16-day-old potato-dextrose-agar cultures containing agar, mycelium, conidia, and conidiophores with a flamed scalpel and mixing the kernels thoroughly with the mass until every grain was coated with the agar bearing the fungus. The other 50 kernels planted in each of the compartments for a control were mixed with the same amount of sterile potato-dextrose agar. After planting, the kernels were covered with soil three centimeters deep, and sufficient water was added to bring the soil moisture to 6.25 percent of the water-holding capacity. Loose-fitting glass covers were placed over the cans to prevent too rapid evaporation of the soil moisture. The experiment was started on February 22, and the final record was taken on March 5



for the seedlings at soil temperatures of 36°, 32°, 28°, and 24° C., on March 15 for the seedlings at 20° C., and on April 4, 1923, for those at 16° C.

The percentage germination in the checks gradually increased with the rise in soil temperature. A similar, although greater, increase in the percentage of germination occurred in the inoculated series. Likewise, the percentage of seedlings free from infection increased with the rise in soil temperature. The percentage of seedlings with mesocotyl lesions increased up to 28° C., and gradually decreased towards 36° C. The coleoptile infection occurred at 16° C. in one of the checks, and at 20° C. in the inoculated lots. The percentage of seedlings with lesions on the seminal roots increased with the rise of soil temperature. The mesocotyl infection, although very abundant at the higher soil temperatures, did not damage the seedlings appreciably, as shown in Plate XXXII. This was because the permanent roots soon form at the first node above the mesocotyl, and the growth of the plants at temperatures from 24° to 36° C. is very rapid compared with the growth of *H. oryzae* within the tissues of the mesocotyl. After the formation of permanent roots, the mesocotyl and the seedling roots are of little value to the seedlings. At lower temperatures the seedlings grow very slowly as compared with *H. oryzae*, and the fungus has therefore sufficient time to spread within the infected regions of the seedlings. As a result, either the mesocotyl, coleoptile, or root lesions are often fatal, because the fungus, though growing slowly, is comparatively more rapid than the growth of the seedlings in its invasion of the organs which are in process of formation.

The most serious form of the Helminthosporium disease is the blighting of the seedlings which occurred most severely at the lower soil temperatures. The blighting of seedlings before they emerged occurred in the checks at 16° and at 20° C., with greater severity at 16° C. In the inoculated cans, blighting before emergence occurred from 16° to 28° C., with greater severity at the lower soil temperatures. In both the checks and the inoculated cans, some seedlings blighted after emergence at soil temperatures from 16° to 32° C. The relative stand of the seedlings in the checks and inoculated series at the different soil temperatures (Plate XXX) shows that less than one half of the seedlings from 20° to 28° C., and none of the inoculated kernels at 16° C., emerged.

In the experiments to determine the relation of type of germination of rice at different soil temperatures to the production and severity of infections by *H. oryzae*, observations were made on seedling vigor at different soil temperatures and also on the length of the coleoptile, mesocotyl, and seminal roots. Schroeder (12), working on the influence of external factors upon the length of the coleoptile of rice and other Gramineae, states that no doubt in many cases temperature had a marked influence upon the length of the coleoptile. The seedlings free from infection by *H. oryzae*, but smaller in size than the rest of the healthy plants because they were in some

way distorted, are designated in this paper as "weak seedlings." These have been noted in the checks from 16° to 20° C., but not at higher temperatures. The coleoptile length followed almost the same curve as length of the mesocotyl at the different soil temperatures. Since the coleoptile remains in more or less close contact around the plumule of the seedling for a relatively longer time at the lower soil temperatures, *H. oryzae* has sufficient time to penetrate through the coleoptile and to invade the embryonic tissues within the coleoptile. At the higher soil temperatures the coleoptile curls out, dries, and finally rots in a comparatively short time, so that the fungus does not have sufficient time to cause infection of the sheaths and blades of the new leaves. The length of the mesocotyl in seedlings planted three centimeters deep increased from 16° to 20° C., and then remained about the same up to 32° C. At 36° C. there was again a slight decrease in length of the mesocotyl. The roots of the seedlings increased in their relative lengths from 16° to 20° C., and from this temperature there was a gradual decrease in length towards 36° C.

The second experiment was started on March 10 and completed on April 4, 1923. The same soil held at the same moisture content as used in the previous experiment was used for the second experiment. Two lots of 50 kernels each of 5891 F6 Binicol rice were planted in each can, separated by means of glass plates as previously described. The 50 kernels in the checks were mixed with one half of a petri dish of finely cut sterile potato-dextrose agar. The other 50 kernels were inoculated with one half of a petri dish each of 32-day-old potato-dextrose-agar cultures of the Louisiana strain AIS of *H. oryzae*. The grains were then covered with three centimeters of soil and the moisture was brought up to 6.25 percent of the water-holding capacity as in the first experiment.

In the second experiment the percentage of germination in both the checks and the inoculated series increased with the rise in soil temperature. Likewise, the percentage of healthy seedlings in both the checks and the inoculated cans increased with the rise of soil temperature. In the checks, the mesocotyl infections gradually decreased in number from 20° to 36° C., but in the inoculated series there was an increase in mesocotyl infections with the rise in soil temperature. Coleoptile infection occurred in the inoculated series at 20° and at 28° C. It was less severe at the latter temperature. Seminal root infection occurred in the inoculated plants only at 28° C. Some blighting before emergence occurred in the checks at 16° C. In the inoculated series, however, blighting before emergence was severe at the lower soil temperatures and gradually decreased with the rise in temperature to 28° C., beyond which no blighting occurred. There was one exception to this rule, however, for at 24° C. blighting before emergence was less than at 20° and 28° C. Weakened seedlings were present in the checks and inoculated series from 16° to 28° C., but gradually decreasing in number with the rise in soil temperature.

The increase in length of roots of the seedlings followed the curve for length of shoot development as shown in Plate XXIX and in the checks in Plates XXX and XXXI. They were longest at 28° and 32°, followed by those at 24°, 36°, 20°, 40°, and 16° C. respectively. The length of the mesocotyl increased at soil temperatures of 20° and 24° C., and gradually decreased from 28° to 36° C. At 32° C. the mesocotyl was shorter than at either 28° or 36° C.

The third and fourth series of experiments were started at the same time. In these two series 100 kernels of the Philippine variety of rice 6040 F6 Kinagaykay were planted in the same manner as described for the first and second experiments. The soil used was sterilized garden loam with 20 percent moisture and having a water-holding capacity of 28.3 percent. After planting, the soil moisture was brought up to 18 percent of the water-holding capacity. The same method of planting and of inoculating which was followed in the first two experiments was used in the third and fourth. The same relative quantity of sterile potato-dextrose agar as that used in the first two tests was mixed with the kernels for the controls. The kernels for the inoculated series were mixed with minced agar from 14-day-old potato-dextrose-agar cultures of the Louisiana strain AIS of *H. oryzae*.

In these two series, the germination in the checks was practically perfect at all soil temperatures. The percentage germination in the inoculated series increased with the rise in soil temperature. Mesocotyl infection occurred in the inoculated series from 16° to 36° C., increasing in prevalence up to 24° and 32° C. respectively in the two experiments and decreasing towards 36° C. The severity of the coleoptile infection decreased with the rise of soil temperature to 32° C.; at 36° C. no coleoptile infection was found in either of these experiments. Seminal root infections occurred from 16° to 32° C., decreasing in number and severity with the rise in soil temperature. Blighting before emergence occurred from 16° to 32° C., decreasing in amount and severity as the temperature increased. Blighting after the seedlings emerged occurred to a limited extent only at 28° and 32° C. The weak seedlings decreased in number with the rise in soil temperature from 16° to 32° C. The length of the roots and of the mesocotyl followed the curve of subsequent development of the aerial parts of the seedlings. The greatest height of the seedlings at the duration of the experiment was at 28° to 32° C., followed by a decrease at 24°, a further decrease at 36°, then at 20°, then at 40°, and finally at 16° C. The length of the seedling roots at different soil temperatures followed the same order as for the shoots, as shown in Plate XXIX and in the checks in Plates XXX and XXXI. The length of the coleoptile remained almost the same at the different soil temperatures, except at 28° C. where it was slightly shorter.

The types of infection of rice seedlings by *H. oryzae* and the ratio of infected plants to the healthy plants of the checks are shown in Plate XXXI, in which five seedlings from the inoculated compartments are shown along-

side five others from the checks. At 16° C. the seedlings were blighted as soon as they germinated. From 20° to 28° C., the seedlings were comparatively smaller than those of the check on account of the infection. Blighting before emergence gradually decreased with the rise in soil temperature. At 28° C. there was no blighting of the seedlings, and at 32° C. and 36° C. the stand of the seedlings in the inoculated and in the check lots was nearly the same. Except for mesocotyl and coleoptile infections at 32° and 36° C., which were not severe at these temperatures, the seedlings were equal in size and vigor to those of the check. The general appearance of the infected seedlings is shown in Plate XXXII, where one healthy seedling from the check (C) is shown for comparison with the infected seedlings (I). The infected seedlings show brown lesions on the mesocotyl, indicated by the arrow heads. At 32° and 36° C., the seedlings were of the same size as those of the checks except that there were brown lesions on the mesocotyl.

From the results of the experiments it was noted that, although rice germinates more rapidly at 36° C., subsequent growth is best from 28° to 32° C. At 40° C. rice germinates at nearly the same time as at 24° or 20° C., but only a few of the seedlings appear above ground. Moreover, the seedlings at 40° C. are pale yellow, because of a deficiency of chlorophyll. The seedlings gradually turn brown and finally die. At 36° C., especially during the summer months, tipburn is produced on the leaves of the seedlings. At 16° C., and to some extent at 36° C., there is a bleaching of the leaves similar in appearance to that at 40° C. The soil moistures used in the experiments were 16, 20, 25, 68.4, 75, and 100 percent respectively of the moisture-holding capacity of the soil. Before the soil became thoroughly saturated, the amount of moisture did not materially affect the time of emergence. As soon as free water was present on the surface of the soil, germination was retarded from one to several days.

The results of experiments conducted to determine the effect of sterilization by heat at a high pressure did not show that heating the soil was injurious to rice at germination. The rice germinated at the same time in both sterilized and unsterilized soil at any given soil temperature. It was shown, however, that the quality of the seed and the influence of the soil temperatures upon it affected the percentage of germination. From 28° to 36° C. the influence of the quality of seed on germination and growth was not so pronounced.

The results obtained in the soil-temperature experiments may be summarized as follows:

1. Certain upland and lowland varieties of Philippine rice when planted one to three centimeters deep in soils having moisture contents of 16, 20, 25, 68.4, 75, and 100 percent of the water-holding capacity emerged in 1 to 4 days at 36° and 32° C., 4 to 7 days at 28° C., 5 to 9 days at 24° C., 6 to 13 days at 20° C., 2 to 6 days at 40° C., and 9 to 16 days at 16° C. When the grains were planted deeper than one centimeter, there was a corresponding

increase in the length of the coleoptile and mesocotyl. On account of this increase in length, there was produced a greater surface for *H. oryzae* to penetrate and thus cause infection. Since the development of the seedling organs was relatively slower at lower soil temperatures, a greater chance was given for the fungus to invade the tender tissues of the seedlings at the lower soil temperatures than at the higher ones. At the lower soil temperatures the seedlings were crisp and brittle. Unlike those at temperatures from 28° to 36° C., the seedlings at the lower temperatures were also very succulent. These conditions were indicative of tissues favorable for invasion by the parasite.

2. Infection of seedlings from pure cultures of the Philippine PO, the Louisiana AIS, and the Japanese JNSr strains of *Helminthosporium oryzae* occurred at soil temperatures from 16° to 36° C. At the lower soil temperatures the lesions were more numerous and the percentage of diseased seedlings was greater. Blighting of seedlings before emergence occurred at temperatures from 16° to 24° C., thus naturally reducing the stand. Perhaps because of the rapid growth of the rice and of *H. oryzae* at temperatures from 28° to 32° C. (10, 11), the development of the superficial lesions was more rapid, and the lesions on the aerial parts of the young plants were larger. Since the coleoptile, mesocotyl, and seedling roots did not become permanent parts of the grown rice plant, and since the development of permanent roots and sheaths from the node above the mesocotyl at temperatures of 28° to 36° C. was very rapid, the progress of the disease at the higher soil temperatures did not keep pace with the growth of the plants.

3. At 36° C. the infection was light and the lesions did not progress as rapidly as at 28° and 32° C. At this temperature tipburn was often produced on the leaves, and to some extent the chlorophyll was destroyed.

4. At 40° C. rice may germinate, but the seedlings did not grow more than a few centimeters before the leaves began to lose the green color, turned brown, and finally died.

5. On account of the short time required for germination at 36° C. and at 32° C., and of the fact that only occasional blighting occurred at these temperatures, it seems probable that seeding on badly infested grounds during hot days when the soil temperatures range from 32° to 36° C., or starting the seedlings in seed beds with temperatures of about 36° C., may reduce infection from *H. oryzae* which overwinters in the soil, in rice stubble, and within the rice grains.

#### THE RELATION OF AIR TEMPERATURES TO THE INFECTION OF RICE SEEDLINGS BY *HELMINTHOSPORIUM ORYZAE*

In the experiments reported previously, the soil temperature was controlled but all plants were growing at the same fluctuating air temperature. Consequently, experiments were conducted to determine the relation of air temperatures to the infection of rice seedlings, and thus to check more

nearly the conditions existing in the field environment. For this experiment, rice grains were germinated in three-inch pots on the greenhouse bench. The seedlings were inoculated with a suspension of conidia applied by means of an atomizer when the shoots were about five centimeters tall. After inoculation, both the inoculated and the check pots were placed in the cans in the temperature chambers. These cans had five centimeters of water at the bottom to supply water to the roots and to the air about the leaves of the seedlings. The cans were covered with sheets of glass which were slightly raised on one side to allow aëration and to permit light to enter the cans.

In the first trial, on September 6, 1921, the seedlings, as they emerged, were sprayed with one atomizer-full of conidial suspension, the conidia of which were taken from a tube of a 21-day-old culture of the Louisiana strain AIS of *H. oryzae*. After 24 hours, the minute, circular to oblong lesions were already apparent on the aërial parts of the seedlings. The result showed that the aërial portions of rice were infected at air temperatures from 16° to 36° C. From 28° to 32° C., at which temperatures rice displayed the best subsequent development, a few large lesions were produced.

In the second experiment, kernels of the variety 5891 F6 Binicol were planted in four-inch pots containing sterilized soil on December 17, 1921, and the seedlings emerged on December 25, 1921. The pots were placed in the cans in the tanks, the bottom of which contained water, on December 28, 1921. The seedlings were atomized with a suspension of conidia prepared from a 21-day-old potato-dextrose-agar culture of the Japanese strain JNS1 of *H. oryzae* until minute droplets containing conidia completely covered the leaves and culms of the seedlings. The duplicate set was atomized in the same manner with a culture of the Louisiana strain AIS of the same age. The third set was used as a check and was atomized with sterile distilled water. The lesions on the leaves and stems of the seedlings appeared in 20 hours in both of the inoculated series. It was noted in connection with the experiment that at 16° C. the lesions were hardly visible, and at 20° C. they were a little larger. At 24° C. the lesions were as abundant as those at 20° and slightly larger. The lesions at 28° C. were still larger than those at 24° C. At 32° C. the lesions were fewer than at 28° C., but they were as large. At 36° C. the lesions, besides being fewer in number, were as minute as at 16° C. At 40° C. the seedlings did not increase in height and there were no lesions produced.

The results show that infection of aërial parts of seedlings by *H. oryzae* occurred at air temperatures of 16° to 36° C. The most favorable temperatures for the development of the lesions were from 28° to 32° C.

As it was not possible to control the moisture content of the air in the cans submerged in the temperature tanks, the inoculation experiments with the aërial parts of the seedlings were continued in controlled temperature and moisture chambers. The moisture content of the air in the chambers



throughout the experiments averaged 80 to 90 percent of the relative humidity. The evaporation coefficients in the different chambers were as nearly the same as it was possible to obtain with the regulation equipment used. The average variation was less than 10 percent. The first chamber was operated at a temperature of 15° C., the second at 29°–30° C., and the third at 36° C. The mechanism and method of operation of these chambers were described by Johnson (3). On February 15, 1922, grains of 5891 F6 Binicol rice were planted in sterilized soil from the wood lot described at the beginning of this paper. Three days later the pots were set in a pan of water to saturate the soil, which had a water-holding capacity of 40 percent. The seedlings emerged six days after planting. On the seventh day, five pots were placed in the chamber the temperature of which was 36° C., five in the chamber with a temperature of 29°–30° C., and five in that at 15° C. The best subsequent growth of the seedlings after five days occurred at the air temperature of 29°–30° C. At 36° C. severe tipburn occurred. On the other hand, the temperature of 15° C. was too low for good growth of the Philippine variety of rice tried.

In order to determine the effect of air temperatures upon the germination of rice and infection by *H. oryzae*, nine four-inch pots were filled with sifted soil from the wood lot having a moisture-holding capacity of 40 percent. The pots were set in a pan of water to saturate the soil. Fifty kernels of 5891 F6 Binicol rice were sown in each pot in such a manner that the kernels were not touching each other. The grains were not buried deeply, but were placed just below the level of the soil surface. After planting, three pots were inoculated on the soil surface with macerated dead leaves of rice containing abundant conidia of the Japanese strain JNS1 of *H. oryzae*, three with macerated dead leaves containing conidia of the Louisiana strain AIS, and the other three, which were used as checks, were inoculated with macerated dead leaves without the fungus. One pot of each of the three sets was placed in each of the three chambers on February 27, 1922.

At 36° C. the rice emerged on March 1, 1922, one day in advance of the grains planted at 29°–30° C. At 36° C. the seedlings in the check were 25.0 centimeters high, in the pot inoculated with the Japanese strain 18.0 centimeters, and in the pot inoculated with the Louisiana strain 20.0 centimeters high. In the chamber maintaining a temperature of 29°–30° C., the seedlings in the check pot and in each of the inoculated pots were 28.0 centimeters tall. As the kernels planted in pots placed in the 15° C. chamber did not emerge when the data were taken, the infection was recorded only at temperatures of 29°–30° C. and 36° C. Four percent of the plants were infected with the Japanese strain JNS1, and six percent with the Louisiana strain AIS at 29°–30° C. No infection with either strain occurred at 36° C. The rice did not emerge even after two weeks at 15° C., although the grains were still alive. When these pots were removed to



the greenhouse bench at room temperature, the seedlings emerged but those in the inoculated series were blighted.

In the next experiment, conducted on March 3, 1922, the same size of pots and the same kind of soil were used as in the preceding experiment. Three of the pots were planted as checks with 50 kernels each of 5891 F6 Binicol rice without inoculation. Three pots were planted with 50 kernels each which had been moistened with water and rolled on a slant of a 20-day-old potato-dextrose-agar culture of the Japanese strain JNS1 so that the rice grains were coated with conidia. The last three pots were planted with rice rolled on a culture of the Louisiana strain AIS of the same age and growing on the same medium. The pots were placed one in each of the three chambers.

In this trial the 5891 F6 Binicol variety of rice emerged in two days at 36° C., and in three days at 29°–30° C. At 36° C. the Louisiana strain produced infection in 20 percent of the seedlings, and the Japanese strain in 18 percent. At 29°–30° C., both of these strains produced infection in 56 percent of the seedlings. At this temperature, many of the seedlings were only five to ten millimeters tall, and many were distorted and blighted. Infection was more severe at 29°–30° C. than at 36° C. The seedlings did not emerge in the pots in the chamber having a temperature of 15° C., and on the eighth day the pots were removed to the greenhouse bench at room temperature. Under this condition the rice emerged, but the seedlings were severely blighted as a result of having remained at 15° C. for eight days. The checks in all cases remained healthy.

The results obtained in the experiments in constant-temperature and moisture chambers showed that rice germinated and emerged quicker at 36° C., but the subsequent growth of the seedlings was better at 29°–30° C. There was a more severe infection of the seedlings at 29°–30° C. than at 36° C. Although the pots were not allowed to remain in the 15° C. chamber until the seedlings germinated and emerged, it was noted that the rice kept in this chamber for a few days was severely infected, perhaps in consequence of a weakened condition resulting from the exposure to the low temperature.

Since the results obtained in the constant-temperature and moisture chambers, where the temperature and moisture of the air over the seedlings were both under control, are the same as those obtained in soil-temperature tanks in which the soil temperature was controlled but not the air temperature and humidity, the writer believes that soil temperature has a greater influence in the production and severity of the disease caused by *H. oryzae* in the seedling stage than air temperature.

#### DISCUSSION

Since rice grows in tropical and subtropical countries, it develops at a higher temperature than most temperate-region crops. It was thought

that, if the parasitic *Helminthosporium* grew best at a somewhat lower temperature, its destructive tendencies could be inhibited or reduced by sowing rice during the warmer part of the year. In experiments to determine the effect of different soil and air temperatures on germination and subsequent development of rice seedlings and on the degree of infection by *Helminthosporium oryzae*, it was noted that the curves plotted to indicate the growth of the fungus and of the rice almost coincide. The optimum temperature for the development of the fungus from Louisiana, Japan, and the Philippines in pure culture lies at about 28° C. The minimum lies at about 16° C. and the maximum at about 40° C. Although Philippine upland and lowland rices germinate much faster at 36° C., the subsequent development of the seedlings is best at 28° to 32° C. The highest temperature is about 40° C. It takes the seedlings from 2 to 6 days to appear above ground at 40° C. The seedlings do not usually remain green at 40° C., but turn yellow, then brown, and finally die. In infection experiments in soil and air temperatures from 16° to 40° C., at 4° intervals, it was noted that infection takes place at from 16° to 36° C. It was noted also that the severity of infection is greater at lower than at higher temperatures. The development of the lesions from primary or secondary infection, however, is much faster at temperatures from 28° to 32° C., owing perhaps to the rapid increase of the rice tissues at these temperatures and to the rapid growth of the fungus. Infection of aerial parts of the seedlings showed that the size of the lesions at 36° C. was the same as that at 16° C., but the spots were fewer in number at 36° C. In soil-inoculation experiments at different temperatures, a higher percentage of infection and more destructive blighting before the seedlings emerge occur at the lower soil temperatures. It was noted that the effect of other seed-borne fungi upon rice germination was extremely limited at 28° C. to 36° C., and as a result better germination of the uninoculated as well as the inoculated series occurred at these soil temperatures.

The influence of soil temperature on the development of the root systems of rice seedlings is similar to the influence on shoot development. Roots were longest at 28° to 32° C. followed by those grown at 24°, 36°, 20°, 40°, and 16° C. The mesocotyl and coleoptile of the seedling are longer when the kernels are planted three centimeters deep than when planted near the surface. There was a slight increase in length of the coleoptile and mesocotyl from 16° to 20° C. From 24° to 32° C., the length remains almost the same as at the lower temperatures. At 36° C. there is again a slight decrease in length.

The blighting of the seedlings due to *H. oryzae* before the seedlings emerge is most severe at 16° C., the lowest soil temperature at which Philippine varieties of rice could be germinated. The severity of blighting gradually decreases, until at 24° C. little or no blighting occurs. The blighting after emergence of the rice seedlings is less severe than the blighting before emerg-

ence. The severity of the blighting after emergence of the seedlings is also greatest at 16° C. and gradually diminishes with the rise in soil temperature, until at 32° C. no blighting occurs.

The number of seedlings with infection of the mesocotyl increases with a rise in soil temperature. The number of seedlings with lesions on the seminal roots increases with the rise in soil temperature. The seedlings with infection of the coleoptile are more abundant at temperatures from 20° to 32° C. Local infections of the coleoptile, mesocotyl, and seedling roots are more abundant at the higher soil temperatures, but do not develop beyond a local spot. The coleoptile, mesocotyl, and seedling roots affected turn brown, die, and finally rot. At higher temperatures, the functions of the coleoptile, mesocotyl, and seedling roots are limited in duration. The coleoptile curls outward, becomes dry, and finally rots. The mesocotyl does not increase in size, and as soon as the permanent roots are produced from the first node the function of the mesocotyl becomes insignificant. Since these changes occur in a comparatively shorter time at temperatures from 28° to 36° C., although the progress of the disease from 28° to 32° C. is much faster than at 16° to 24° C., the rice seedlings finally outgrow the disease. At lower temperatures, both rice seedlings and *H. oryzae* grow slowly. Although the growth of *H. oryzae* is slow at lower temperatures, it is nevertheless relatively faster than the development of the tissues of the rice seedlings at these temperatures. Undoubtedly, the tissues of the slow-growing rice seedlings are different in chemical composition from those making a vigorous growth at the high soil temperatures. Consequently, at the lower soil temperatures rice seedlings afford a greater chance for the mycelium of *H. oryzae* to penetrate the coleoptile and mesocotyl and enter the embryonic tissues of the seedlings. In consequence, even though the infection may be on such temporary organs as the coleoptile, mesocotyl, and seedling roots, the fungus finally invades the embryonic tissues of the seedlings and often causes blighting before emergence. Or, these infections of the coleoptile, mesocotyl, and seedling roots at the lower soil temperatures are often conducive to blighting of seedlings after their emergence. At 28° to 32° C., primary and secondary infections on leaves and sheaths are also outgrown by the rice seedlings.

In constant-temperature and moisture chambers, germination was faster at 36° C. than at 29°–30° C.; but the development of the seedlings after emergence was better at 29°–30° C. It was also noted that in the chamber where the development of the seedlings was better the development of the lesions was faster. After allowing the pots to remain for a few days in the chamber having a temperature of 15° C., the rice became very much weakened by the exposure to the low temperature. In consequence, although the pots were removed to the greenhouse bench at room temperature, many of the seedlings were blighted at emergence. An air temperature of 36° C. retards the development of severe spots on the aerial parts of the seedlings.

In conclusion, therefore, the disease is most severe at temperatures from 16° to 24° C. The most severe form of the disease is the seedling blight. At temperatures from 28° to 36° C., although occasional blighting occurs, the percentage of infection is not high. Consequently, the most severe form of the Helminthosporium disease of rice may be avoided by planting during the warmer part of the year or by starting seedlings in seed beds with temperatures of from 32° to 36° C.

#### SUMMARY

The results of the experiments discussed in the foregoing pages may be summarized as follows:

1. Experiments conducted to determine the effect of soil temperatures from 16° to 40° C., with soil moisture varying from 16 to 100 percent of the water-holding capacity, upon the germination of rice, showed that emergence occurred in from 1 to 4 days at 36° C. and 32° C., in from 4 to 7 days at 28° C., in from 5 to 9 days at 24° C., in from 2 to 6 days at 40°, in from 6 to 13 days at 20° C., and in from 9 to 16 days at 16° C. It was also found that sterilizing the soil for a long time under high steam pressure did not produce much effect upon the rate of germination or the time of emergence. The quality of the seed affects the percentage of germination at soil temperatures from 16° to 24° C. Above this soil temperature, the influence of seed-borne parasites becomes less effective.
2. Inoculation experiments with *H. oryzae* on rice demonstrate that seedlings are infected at soil temperatures from 16° to 36° C. The development of the lesions on the aerial parts of the rice is much faster from 28° to 32° C., because of the rapid growth of the fungus and of rice seedlings at these temperatures. Severe blighting of seedlings before emergence occurs at 16° to 24° C. At 36° C. the percentage of infection is low and the growth of the lesions is comparatively slow.
3. The temperatures favorable for the growth and sporulation of *H. oryzae* and for the growth of Philippine varieties of rice are nearly the same. The development of the lesions is most rapid at temperatures at which the subsequent growth of the rice is best. A more severe infection, resulting in blighting of the seedlings before emergence, occurs at soil temperatures from 16° to 24° C. At 36° C., only an occasional blighting of the seedlings occurs. Planting rice in soil or in seed beds with temperatures of from 32° to 36° C. will materially reduce infection and blighting by *H. oryzae*.
4. Although the coleoptile, mesocotyl, and seedling-root infections are more abundant and the lesions develop faster at higher soil temperatures, they are less harmful and are finally outgrown by the rice seedlings.
5. At lower soil temperatures, even coleoptile, mesocotyl, and seedling-root infections are conducive to blighting after the seedlings have emerged through the soil.

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## DESCRIPTION OF ILLUSTRATIONS

## PLATE XXIX

Uninoculated seedlings of the Philippine variety of rice 9115 F2 Dinalaga, grown at soil temperatures of 16°, 20°, 24°, 28°, 32°, 36°, and 40° C., 10 days after planting, showing the relative sizes of the seedlings. The seedlings are tallest at 32° and 28° C., followed by those at 24° and 36° C. At this time the seedlings at 20° C. are only a little larger than those at 40° C. The rice at 16° C. was barely sprouting. The best subsequent growth is at 28° to 32° C.

## PLATE XXX

Seedlings of the Philippine variety of rice 5891 F6 Binicol, grown at soil temperatures of from 16° to 36° C. The seedlings to the left in each can were checks (C), and those at the right were inoculated (I) at the time of planting. The grains were planted 3.0 centimeters deep. The inoculated and the check grains were separated by glass plates. There was no germination of the inoculated grains at 16° C., and very few of the seedlings emerged at 20° C. At 24° and 28° C., the reduced stands of the seedlings are shown. At 32° and 36° C., no reduction of stand and no visible effect was shown. The results seem to indicate that, in heavily infected soil, seeding during the warmer part of the year or starting seedlings in warm beds will prevent the most serious form of the disease, the seedling blight. In contrast, the seedling blight occurs at the lower soil temperatures, from 16° to 24° C. The series was planted on March 10, 1923, and photographed April 2, 1923.

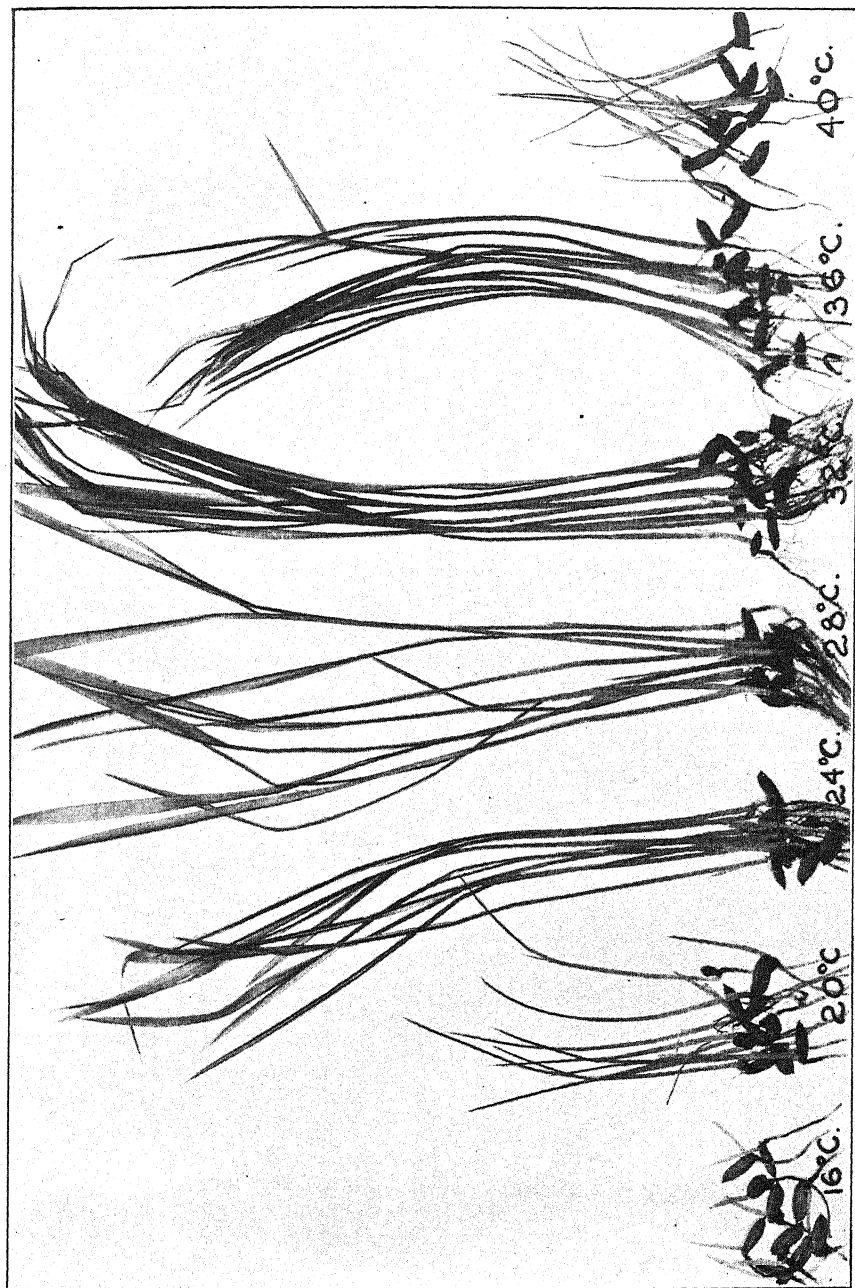
## PLATE XXXI

Seedlings of the Philippine variety of rice 5891 F6 Binicol, showing types of infection at the different soil temperatures. At 16° C., four out of five seedlings shown in the inoculated series (*I*) were blighted before emergence, and one soon after emerging. At 20° C., two out of five seedlings were blighted before emergence, and one was badly infested and weakened. At 24° C., one of the seedlings was badly dwarfed and one was blighted before emergence. At 28° C., one of the seedlings was weakened, while at 32° and 36° C. only mesocotyl and coleoptile infections were produced. The coleoptile and mesocotyl lesions could not be shown distinctly on the reduced photograph. The series was planted March 10, 1923, and examined and photographed April 4, 1923.

## PLATE XXXII

Seedlings of the Philippine variety of rice 6040 F6 Kinagaykay, showing the influence of soil temperature on seedling blight. Only one of the check seedlings (*C*) is exhibited at each of the soil temperatures from 16° to 36° C. Note the brown areas on the seedlings which germinated from the inoculated grains (*I*). At 16° C., all the seedlings were blighted before emergence. At 20° C., two seedlings were blighted before emergence and three had badly infected coleoptiles. At 24° C., two seedlings were blighted and the rest had mesocotyl and coleoptile lesions. From 28° to 36° C., the mesocotyl lesions are indicated by the arrow heads. Note that the presence of the lesions on the mesocotyl from 28° to 36° C. has no serious effect on the vigor or size of the seedlings, as shown by comparison with the seedlings of the check series. The series was planted on March 24, 1923, and examined and photographed on April 5, 1923.

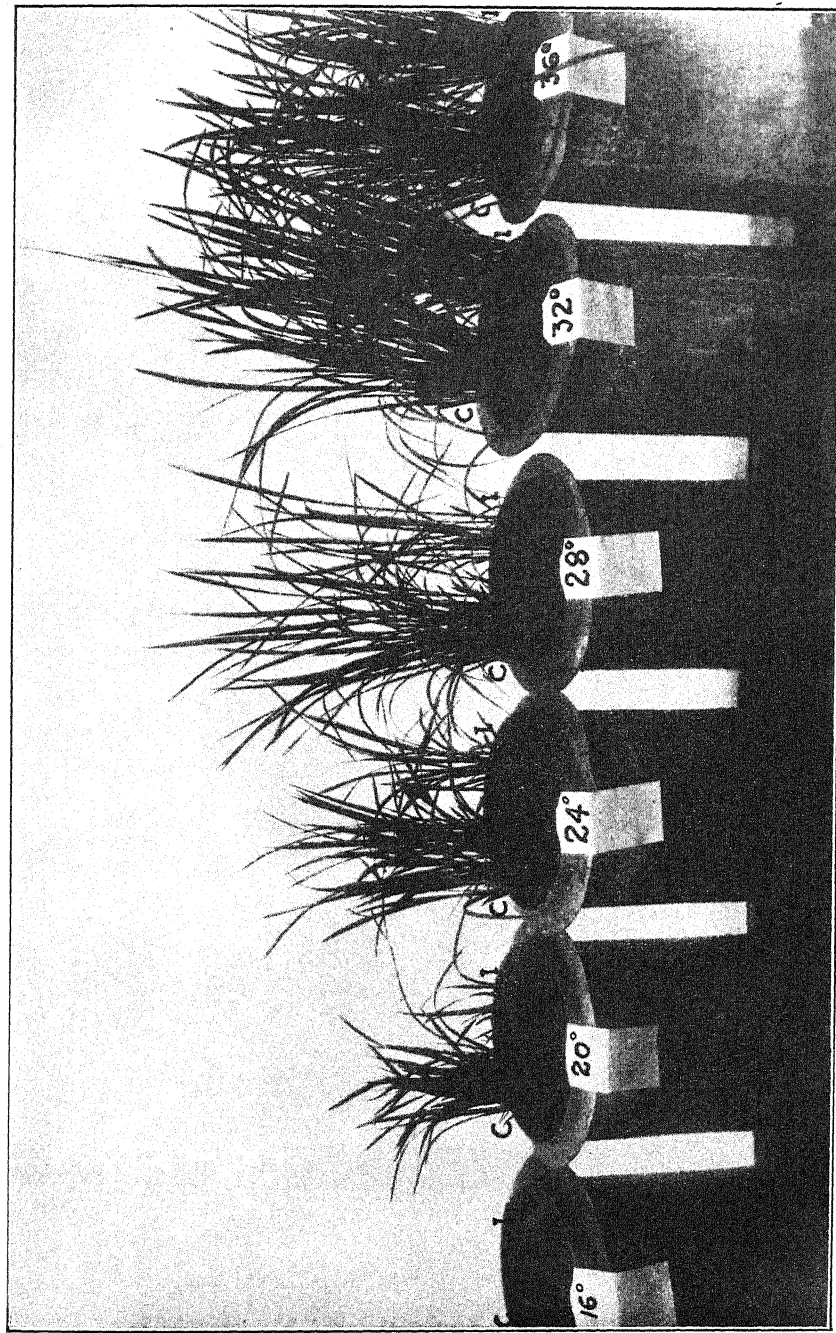




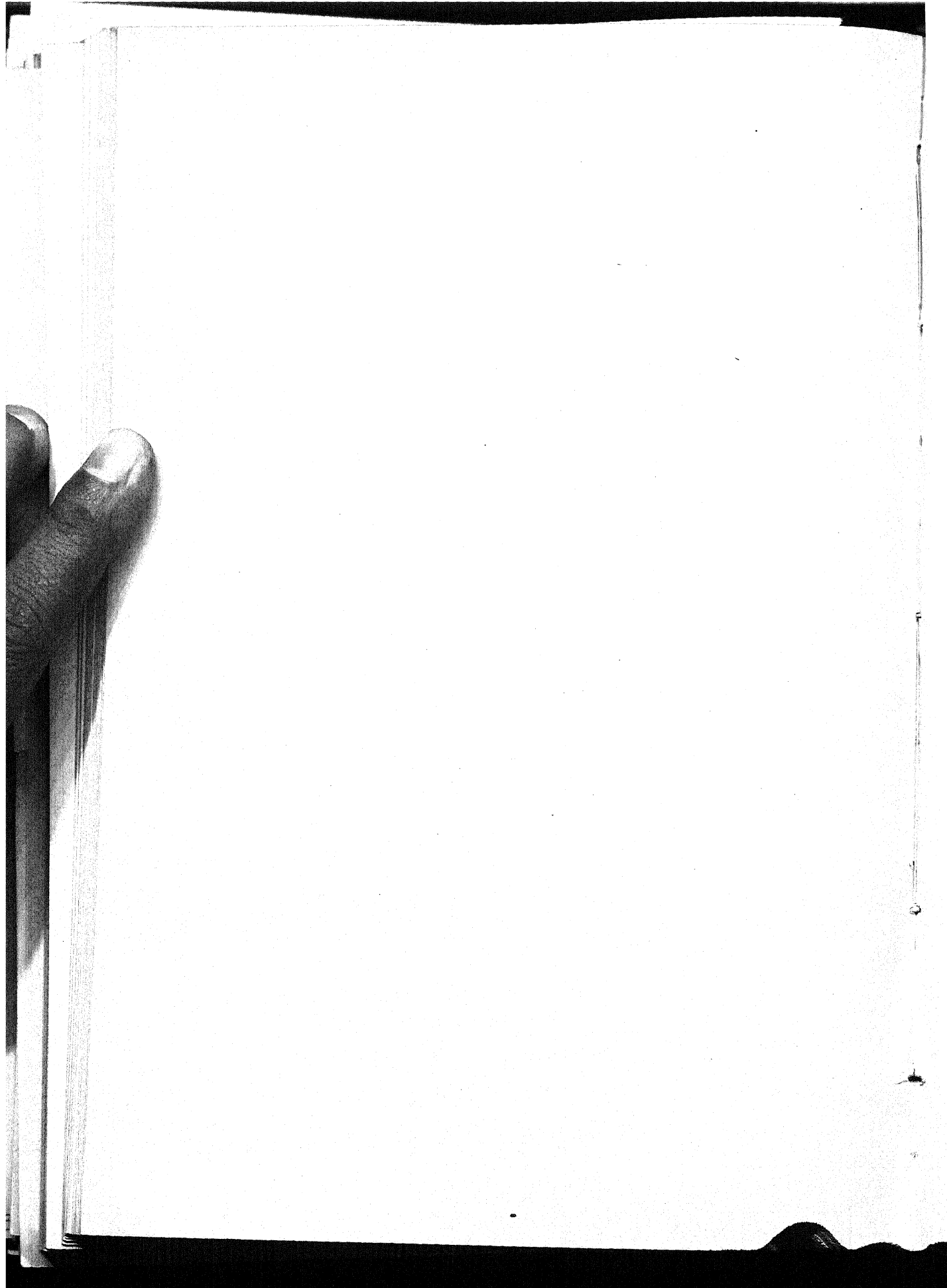
OCEPIA: GERMINATION AND INFECTION OF RICE

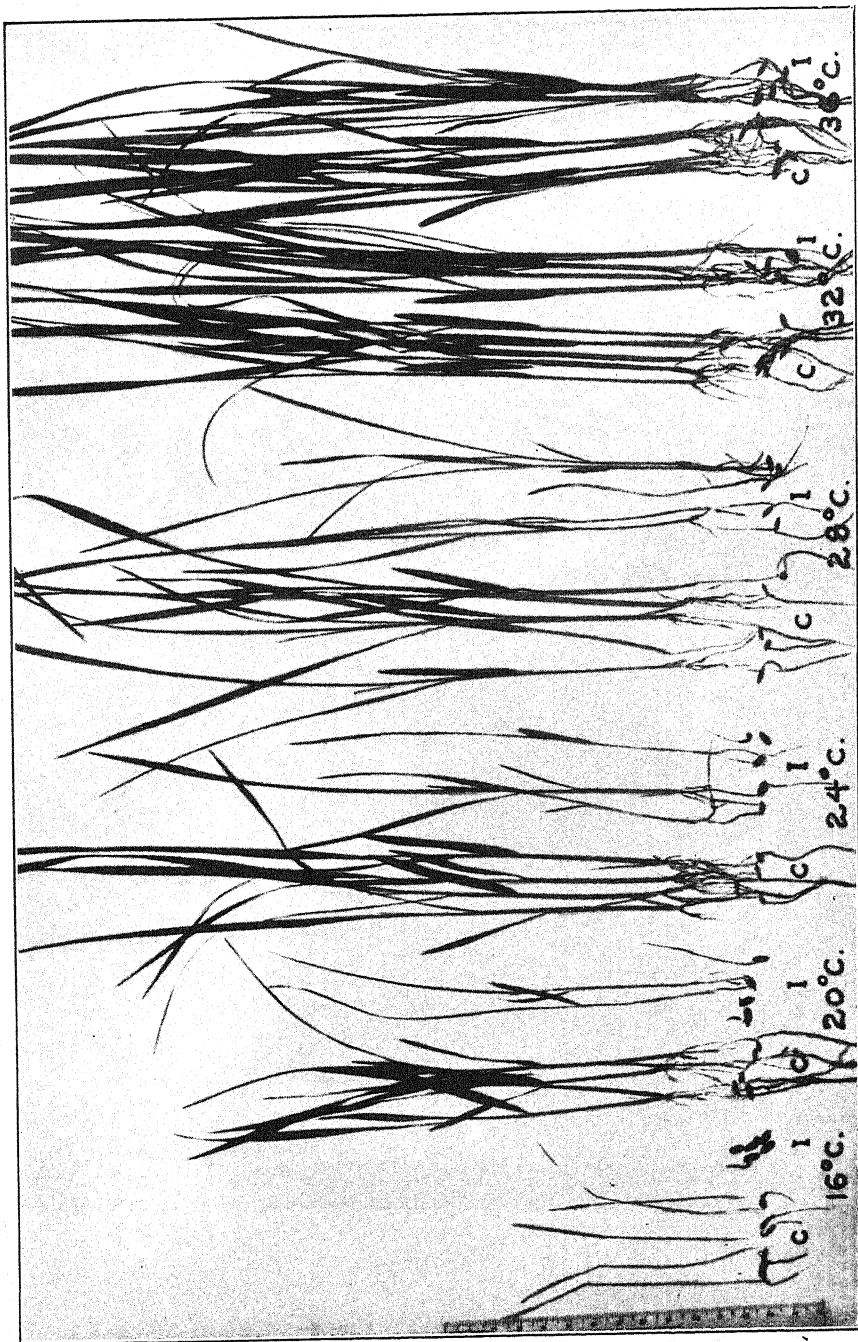






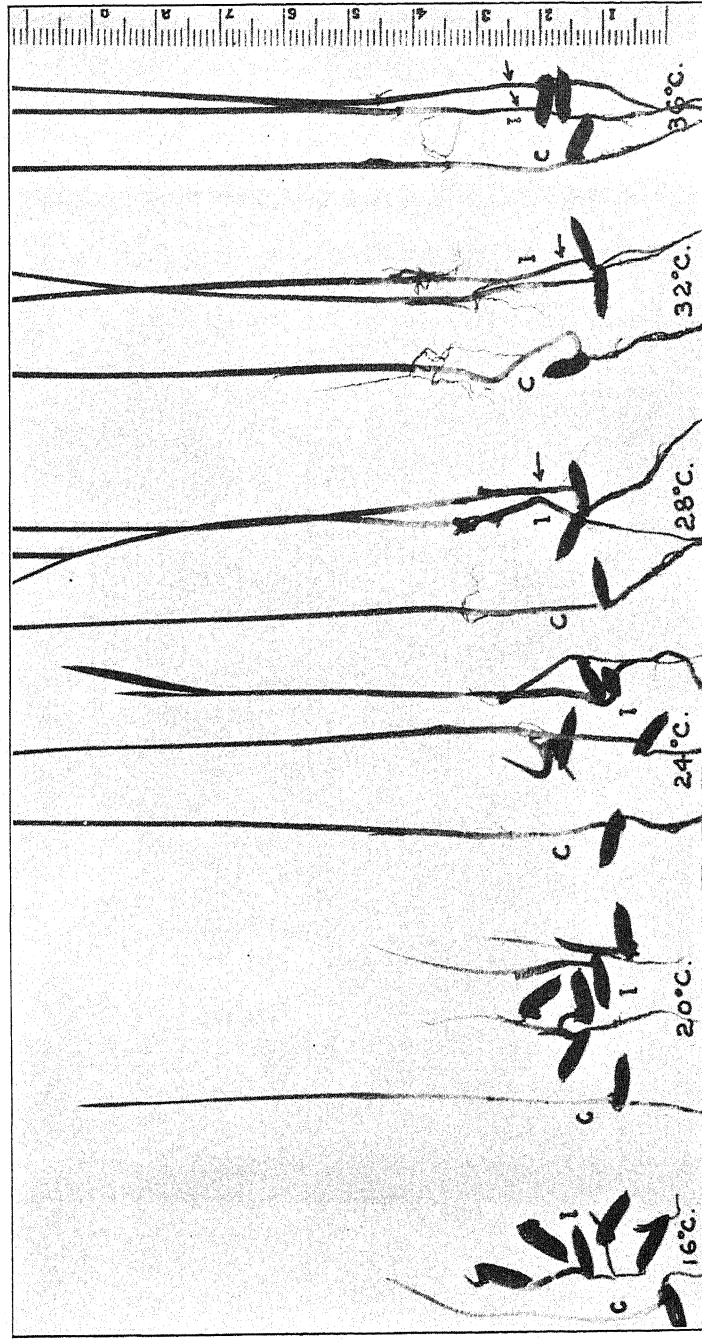
OCEMIA: GERMINATION AND INFECTION OF RICE





OCEMIA: GERMINATION AND INFECTION OF RICE





OCFEMIA: GERMINATION AND INFECTION OF RICE







## A CASE OF POLLEN DIMORPHISM IN MAIZE<sup>1</sup>

M. DEMEREC

(Received for publication September 22, 1923)

Few characters that are expressed in the gametophytic generation of higher plants, and therefore determined by the haploid number of genes, have been described. The inconspicuousness of the gametophytic generation and the difficulty of making observations are probably mainly responsible for this. The few cases described, however, indicate that gametophytic characters do not differ widely from sporophytic characters.

### REVIEW OF LITERATURE

Watt (1907) noted in  $F_1$  plants of crosses between different species of *Gossypium* that one and the same androecium possessed invariably two or more forms of pollen grains. The grains differed in size and shape, and had the characteristics of the pollen grains of either the parental or other species of *Gossypium*. He observed, for example, in  $F_1$  plants from a cross of *G. goghari* X *G. varadi*, three kinds of pollen, which had the characters of the pollen of *G. obtusifolium*, *G. Nanking*, and *G. hirsutum*. No attempt was made, however, to ascertain the relative proportions of each form of pollen present, but the observation of several definite forms in the same pollen sac on an  $F_1$  plant suggests segregation of gametophytic characters.

Belling (1914) made a genetic analysis of semi-sterility of  $F_1$  crosses between species of *Stizolobium*. He found that fifty percent of the pollen grains of  $F_1$  hybrids lose their living contents while in the vacuolated stage, and that fifty percent of the embryo sacs abort long before maturity. After carrying the test through five generations, he concluded that two factors are responsible for the non-development of microspores into perfect pollen grains and of functional megaspores into perfect embryo sacs. In this case, apparently, the effect of two gametophytic factors could be observed on male and female gametes.

Correns (1917, 1918, 1921) analyzed a gametophytic character which affects the rate of pollen-tube growth in *Melandrium*. The effect of this character is observed in the change produced in the sex ratio. This change in sex ratio was not obtained in all crosses (Correns, 1921), which fact suggests that slow growth of the pollen tube is produced by a sex-linked factor.

Renner (1919a, 1919b) described segregation in size and shape of pollen grains, in size and shape of the starch grains in the pollen, and in the rapidity of pollen-tube growth in the  $F_1$  generation of different *Oenothera* crosses.

<sup>1</sup> Paper No. 112, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

Parnell (1921) analyzed a simple Mendelian character in rice which could be detected both in the sporophytic and in the gametophytic generations. In both generations the reserve food is changed from starch to amyloextrin. In the sporophyte, the character is observed in the endosperm of the seeds, and in the gametophyte it is seen in the pollen grains when made visible by the iodine test.

#### EXPERIMENTAL RESULTS

The gametophytic character observed in maize is parallel to that found by Parnell (1921) in rice.

Collins (1909) described a type of maize obtained from China, which had a peculiar waxy texture of endosperm. Since that time it has been shown (Collins and Kempton, 1911) that waxy endosperm is a simple Mendelian recessive to starchy endosperm.

Weatherwax (1922) found that waxy endosperm turns red when treated with iodine and concluded that erythroextrin rather than starch is the carbohydrate characteristic of the endosperm of waxy maize.

Pollen from a homozygous starchy plant and from a homozygous waxy one was examined in a solution of iodine and potassium iodid in dilute alcohol. All pollen grains of the starchy plant turned blue, and the pollen of the waxy plant was colored reddish. Examination of pollen from plants heterozygous for waxy revealed that in one and the same anther both types of pollen were present in approximately equal numbers. Table I gives the results of pollen counts of four anthers taken from the same plant.

TABLE I

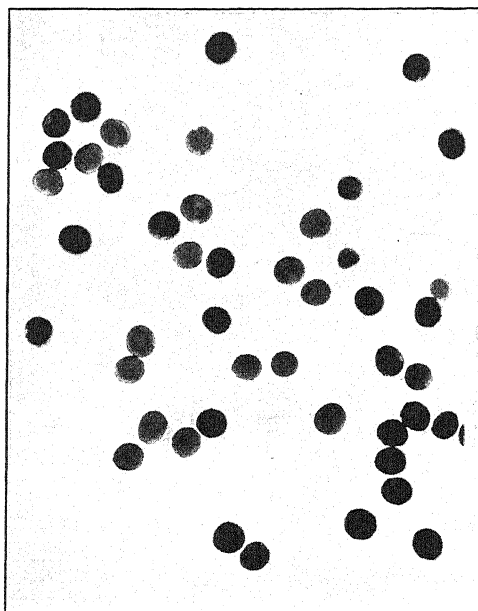
Anther	No. of Pollen Grains			Calculated 1 : 1	Difference
	Purple	Reddish	Total		
1	867	900	1,767	883.5	16.5 $\pm$ 14.2
2	596	602	1,198	599.0	3.0 $\pm$ 11.7
3	929	916	1,845	922.5	6.5 $\pm$ 14.5
4	1,045	1,064	2,109	1,054.5	9.5 $\pm$ 15.4
Total	3,437	3,482	6,916	3,459.5	22.5 $\pm$ 28.0

A microphotograph of pollen taken from a single anther and treated as noted above is reproduced in text figure 1.

Many more anthers were examined, but counts were not made. All observations made have shown an apparently equal mixture of the two kinds of pollen.

To make sure that the plant used in these investigations was heterozygous for waxy, its silks were pollinated with pollen from a homozygous waxy plant. Fifty percent waxy and fifty percent starchy seeds were obtained, just as was expected.

The pollen of several other plants heterozygous for waxy was examined, and in every case it was found that the two types of pollen were present in approximately equal numbers in each anther examined.



TEXT FIG. 1. Microphotograph of pollen taken from a single anther, mounted in a solution of iodine and potassium iodid in dilute alcohol and slightly heated. The waxy grains appear light and the starchy ones dark in the photograph. Photograph by Dr. Ernest F. Artschwager.

#### SUMMARY

It has long been known that waxy endosperm of maize is a simple Mendelian recessive to starchy endosperm. As shown by Weatherwax (1922), erythrodextrin is responsible for the waxy texture.

By treating the pollen of heterozygous plants with iodine, it was found that segregation could be detected in the gametophytic generation. Plants heterozygous for waxy endosperm had about fifty percent waxy and fifty percent starchy pollen grains in one and the same pollen sac.

The case is parallel to that described by Parnell (1921) for rice.

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# SEGREGATION FOR THE WAXY CHARACTER IN MAIZE POLLEN AND DIFFERENTIAL DEVELOPMENT OF THE MALE GAMETOPHYTE<sup>1</sup>

(Received for publication September 22, 1923)

R. A. BRINK AND J. H. MACGILLIVRAY

Random fertilization among compatible gametes is one of the cardinal principles upon which the Mendelian theory of inheritance turns. The array of breeding facts on which the concept rests is so extensive, and the approximations to expectation on the chance basis are so satisfactory in almost innumerable cases, that the general validity of the rule would seem to be established. The fusion of male and female nuclei is associated, however, with various morphological features promoting the sexual act. It has been thought that in certain forms these features may impose conditions upon the central feature of fertilization tending to disturb the chance relation. The mechanism attending fertilization in the angiosperms is a case in point.<sup>2</sup>

The events leading to fecundation in the angiosperms are unique in that the two sperms contributed by each microgametophyte are dependent for their transference to the embryo sac upon a pollen tube whose development is governed by a third member, the vegetative or tube nucleus. In its development the pollen tube traverses the style, a commonly elongated structure connecting the receptive surface with the ovary. The tube nucleus is identical in its genetic constitution with the sperm nuclei accompanying it, but takes no part presumably in fertilization proper; but, as is evidenced by its larger size and its position in the most active region, it dominates the metabolic processes of the gametophyte. In contradistinction to the passive sperm nuclei, the vegetative nucleus becomes the centre of the brisk activity characterizing pollen-tube growth. Putting it in figurative terms, the tube nucleus pilots the conveyance in which the gametes are passengers.

This mechanism interposed between segregation and fertilization might offer a basis for some selective action through differential pollen-tube growth if it were a fact that the microspore nucleus and the tube nucleus during their activity realized their hereditary potentialities to a significant degree. There is evidence from a few forms that this is the case. Belling<sup>3</sup> has

<sup>1</sup> Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, no. 38. Published with the approval of the Director of the Station.

<sup>2</sup> In regard to the terminology employed in this paper, the authors are indebted to Dr. C. E. Allen for helpful criticisms.

<sup>3</sup> *Zeitschr. Ind. Abst. Vererbungsl.* 12: 303-342. 1914.

shown that in *Stizolobium* one half the pollen grains of a definite proportion of the plants in each generation regularly abort. More recently, the same author <sup>4</sup> found in *Rhododendron*, where the pollen grains adhere in sets of fours, that in one of the clones examined two members of each tetrad were abortive. These two cases may be considered as evidence of the operation of lethal factors during the gametophytic generation. Parnell <sup>5</sup> has demonstrated that in rice, hybrids between a starchy type and a so-called glutinous variety produce pollen grains of two types in about equal numbers. The reserve materials in endosperm and pollen grain of the starchy race give the blue reaction with iodine characteristic of starch; in the glutinous form the corresponding products stain reddish, indicating the presence of dextrin. Pollen grains giving the blue reaction and those giving the red occur with about equal frequency on cross-bred plants.

On examining the pollen grains of a number of varieties of maize, the authors have recently found that the microspores produced by the waxy race <sup>6</sup> may be distinguished from the others through their failure to give the starch reaction with iodine. The principal reserve material of the endosperm of the waxy race is a dextrin. Weatherwax <sup>7</sup> contends that it is "erythrodextrin," a term of doubtful significance in view of the limited knowledge of the chemistry of the group. In dilute iodine the reserve material of the waxy pollen stains a dull grayish yellow; in stronger solution, or on standing unsealed for several hours, a reddish tint may appear. As does the endosperm, so the pollen of flint, dent, pop, and sweet varieties, with the exception of a very few grains, shows greater or less amounts of starch staining blue with iodine. The method employed in examining the pollen was simple. The grains were placed in a drop of iodine solution on a slide, and after a few minutes a cover glass was put in place and sufficient pressure was exerted on it to burst the pollen grains and to free a portion of their contents from the spore coat. In all cases a sharp differentiation was secured between the waxy and the non-waxy types of pollen reserves.

The facts regarding the nature of the pollen from plants heterozygous for the waxy factor, of which we had three, are of especial interest. Each of these plants produced starchy grains and waxy grains in approximately equal numbers <sup>8</sup> as shown by the following counts: 493:501, 345:390, and 175:190. There is a slight excess of waxy type grains. This is probably to be accounted for by the fact that varying percentages of ill-developed grains giving no reaction for starch occur in all maize plants. Since iodine

<sup>4</sup> Ann. Rept. Dept. Genetics Carnegie Inst. Wash. 20: 110-111. 1921.

<sup>5</sup> Jour. Gen. 11: 209-212. 1921.

<sup>6</sup> The plants carrying the waxy factor used in these studies were grown from a few seeds representative of some material kindly furnished by Dr. R. A. Emerson, Cornell University, for another purpose.

<sup>7</sup> Genetics 7: 568-572. 1922.

<sup>8</sup> According to a recent note by D. F. Jones (Bot. Gaz. 75: 427, 428), Demerec also has observed this phenomenon.

does not clearly differentiate them from the waxy type, they would be included in this class in our counts. Examination of a number of lines of sweet and flint corn showed from one to five percent of such pollen; in a strain of Burr's White Dent obtained from D. F. Jones and inbred for at least six generations, about eight percent of the grains were defective.

The clean-cut qualitative differences that have been demonstrated in pollen grains of hybrid rice and maize plants might provide a chemical basis for selective pollen-tube growth. Such data as are available have been examined with this possibility in mind. Parnell (*loc. cit.*) selfed nine  $F_1$  plants following the cross of the starchy and glutinous varieties of rice. In all, 6879 seeds were obtained of which 1587 were of the glutinous type. According to Parnell, each of the nine plants gave "about the same ratio." On a monohybrid basis, 1720 individuals of the glutinous sort would be expected, or 133 more than were obtained. This deficiency of the non-starchy type is 5.5 times the probable error, and the odds against its being due to chance alone are several thousand to one. Parnell apparently did not regard this wide departure from expectation as being of any particular significance. The facts, however, suggest that there is a selective action of some sort which discriminates against fertilization by male gametes carrying the glutinous factor.

From the results secured by Collins and Kempton,<sup>9</sup> Kempton,<sup>10</sup> and Hutchison,<sup>11</sup> a table has been constructed bringing together in convenient form data on 179,313 individuals resulting from crosses of three types involving maize plants heterozygous for the waxy character. Limitations of space forbid the presentation of this material in detail, but examination of the separate items entering the table leads us to believe that grouping them as we have done is a legitimate procedure.

Source of Data	Type of Cross		
	Wxwx ♀ × Wxwx ♂	Wxwx ♀ × wxwx ♂	wxwx ♀ × Wxwx ♂
	Collins and Kempton (1911) Kempton (1919)	Kempton (1919) Hutchison (1922)	Kempton (1919)
No. of ears . . . . .	198	107	58
Total seeds . . . . .	102,429	50,231	26,653
Waxy seeds . . . . .	24,500	25,298	13,124
Expected waxy . . . . .	25,607	25,115.5	13,326.5
Deviation . . . . .	-1,107	+ 182.5	- 202.5
Dev. ÷ P. E. . . . .	11.84	2.42	3.68

As shown in the first column of figures in the table, the proportion of waxy seeds is short of the twenty-five percent expected when heterozygous

<sup>9</sup> IV. Confer. Intern. Gén. (Paris), pp. 347-356. 1911.

<sup>10</sup> U. S. D. A. Bur. Plant Ind. Bull. 754: 99. 1919.

<sup>11</sup> Cornell Univ. Agr. Exp. Sta. Mem. 60: 1421-1473. 1922.



plants are selfed by 1107, a deviation 11.8 times the probable error. The odds against a deviation of this magnitude being due to chance alone are over a billion to one. In back-crosses where segregating plants were used as pollen parents, there is again a deficiency of waxy zygotes, as given in the last column of the table, equal to 3.68 times the probable error. The odds against this deviation being due to chance are about 75 to 1. As Kempton has pointed out, the deficiency of waxy seeds in both these populations is the result in large part of the accumulation of deficiencies in the sub-groups (distributions from single ears) which when considered individually do not show a significant departure from the expected ratio. As recorded in the middle column of the table, the proportion of waxy seeds among the progeny of heterozygous plants pollinated with pure waxy does not differ significantly from the expected fifty percent.

The breeding facts leave little doubt that, when maize plants segregating for the waxy character are used as pollen parents, there is a significant deficiency of waxy zygotes. Kempton has recognized these facts and has suggested several possible explanations. Perhaps varying numbers of waxy seeds were included in the non-waxy group during classification. From the nature of his material Kempton does not consider this probable. It should be pointed out, moreover, that the breeding facts do not favor such a conclusion. If there were a constant tendency to include waxy seeds in the starchy class, then the excess of waxy individuals should characterize all three groups discussed above. This is not the case; when the homozygous waxy type was used as the pollen parent in matings with heterozygous individuals the deviation is in the opposite direction, although the approximation to equality in the proportion of waxy and non-waxy seeds is fairly close. Kempton suggests further that the deficiency of waxy seeds may be due to the formation of fewer pollen grains carrying this factor. The evidence from pollen-grain counts lends no support to this view.

A third possibility recognized by Kempton refers to differential viability. Such a condition may well be possible in view of the chemical difference we have established in the two types of pollen. An attempt has been made to investigate this point by conducting a germination test, but the results were valueless. The difference to be accounted for is a small one, and the difficulties involved in satisfying the statistical requirements of such an experiment are not to be ignored.

Another explanation quite as plausible, and in reality differing from the preceding one only in degree, relates to the possibility of a differential rate of growth of waxy and starchy pollen tubes. It may reasonably be supposed that the presence of different sorts of reserve material in the pollen of the two races of maize denotes a somewhat different form of metabolism in the male gametophytes. Our tests, while not conclusive, indicate that the principal reserve material in the pollen of waxy plants is a dextrin. As

is well known, the dextrins are closely related to starch, intermediate products in fact between, and associated with, starch and maltose in starch hydrolysis. The difference in metabolism incident upon the presence of two such closely related storage products may indeed be a slight one; but it will be recalled that the deficiency of waxy zygotes is small, so small as to fail to arouse much suspicion in ordinary samples. In accounting for a difference of this magnitude, only a slight alteration in the growth mechanism is demanded.

Perhaps the male gametophyte in angiosperms is so greatly reduced that little opportunity is afforded for the realization of genetic differences among pollen grains. There is considerable evidence to show that *in general* this is the case; and fortunately so, for chaos would certainly result if conditions were such that the style regularly functioned as a gametic sieve. But what are we to infer from those cases in which differences have been demonstrated that apparently do influence the rate of pollen-tube growth? We submit the following as a working hypothesis. The pollen tube, brief as its career may be, is nevertheless the center of a vigorous vegetative activity. This presumably is controlled by the action of certain factors in the tube nucleus. Among pollen tubes whose nuclei differ significantly in these particular factors, corresponding variations may be expected in development. Under the conditions surrounding fecundation in the angiosperms in such cases, limitations may be imposed upon certain types of gametes in their approach to the eggs. In rice and maize respectively it has been demonstrated that a single factor difference may occasion a significant change in carbohydrate metabolism. Cases in which single factors cause conspicuous differences, however, may prove to be rare. Perhaps Jones'<sup>12</sup> striking results with pollen mixtures of certain strains of maize are due to differences in the vegetative nuclei of the respective pollen samples in regard to several factors attaining a certain modicum of expression in the gametophytic generation, individually of small consequence however, but whose cumulative effects cause a measurable difference in pollen-tube growth.

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<sup>12</sup> Biol. Bull. 38: 251-289. Ibid. 43: 167-174.

GENERA OF NORTH AMERICAN FABACEAE II.  
TRIBE GALEGEAE (CONTINUED)<sup>1</sup>

P. A. RYDBERG

(Received for publication October 12, 1923)

SUBTRIBE 6. DIPHYSANAE

Trees or shrubs, with odd-pinnate leaves, caducous stipules, and no stipels. The flowers are borne in axillary racemes, articulated to the pedicels and subtended by two caducous bractlets. The calyx is surmounting an evident hypanthium, 5-lobed, but the lobes unequal, the upper two broadest and united higher up. The pod is stipitate, oblong, the pericarp separating into two layers: the endocarp, which is chartaceous, forming a flattened cell somewhat interrupted between the seeds, and the exocarp, which is papery, reticulate, becoming strongly inflated and forming an elongate bladder along each side of the legume.

13. *Diphysa* Jacq. Enum. Pl. Carib. 7. 1760

Trees or shrubs. The leaves are odd-pinnate, but the leaflets often arranged more or less alternately on the rachis. The calyx-tube is campanulate, surmounting the turbinate or obconic hypanthium below the insertion of the staminal sheath; the uppermost two lobes are broad and rounded at the apex, the lateral two of about the same length but narrower, obtuse or acute, the lowest one narrow, lanceolate or subulate, acute or acuminate, somewhat longer than the rest. The petals are unequal, the banner usually the longest and the keel petals the shortest, all short-clawed; the blade of the banner is reflexed, suborbicular, with two callosities above the base; those of the wings are obliquely obovate, falcate, auriculate at the base on the upper side; those of the keel-petals lunate, strongly falcate, acutish to rostrate, free at the tip, auriculate or angled at the base. The stamens are diadelphous, the upper filament distinct; the anthers subuniform. The seeds are transversely oblong, somewhat oblique, attached near the end to the slender funicle, the radicle incurved.

ILLUSTRATION: Plate XXXIII, *M. Diphysa robinoides* Benth.,  $\times \frac{2}{3}$ :

1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 1$ ; 7. pod, 8. cross section of the same, 9. seed,  $\times \frac{2}{3}$ .

The genus was based on *Diphysa carthagenensis* Jacq.

It consists of 17 species, natives of Mexico and Central America. Of these, one, *D. carthagenensis*, extends into Colombia and another, *D. Thurberi*, into Arizona. The latter has had a rather interesting history. It was first collected by Thurber at Mabibi, Sonora, in flower and described as *Daubentonia Thurberi* A. Gray. As the flower has a well developed hypanthium and the leaves are odd-pinnate instead of abruptly pinnate,

<sup>1</sup> The first paper of this series was published in an earlier issue of this JOURNAL (Amer. Jour. Bot. 10: 485-498. 1923).

its retention in *Daubentonia* has been questioned, and it was transferred to *Ormocarpus* by Benth. Otto Kuntze named it both *Emerus Thurberi* and *Solulus Thurberi*, the former based on *Ormocarpus Thurberi* Benth., the latter on *Daubentonia Thurberi* A. Gray.

#### SUBTRIBE 7. CORYNELLANAE

Shrubs or trees with abruptly pinnate leaves. The flowers are fascicled on short axillary branches. The calyx is campanulate, the tube as broad as long or nearly so. The banner is suborbicular to ovate or obovate, short-clawed, more or less reflexed; the wings are short-clawed with oblong or obliquely oblanceolate blades, distinct, usually with small basal auricles; the keel-petals similar, often broader, usually more lunate, their blades united from the middle to or nearly to the tip. The stamens are diadelphous, the staminal sheath not more dilated below, straight or nearly so, the upper filament free. The style is glabrous, more or less arcuate. The pod 2-valved, flat, linear, sessile or short-stipitate, sometimes slightly torulose, but neither with cross partitions nor breaking up into joints. The seeds are from orbicular to reniform, somewhat flattened, without funiculum.

The subtribe consists of four genera and ten species, all natives of the West Indies.

Calyx truncate, with 5 minute teeth, of which the upper two are close together; banner orbicular.

Stigma subterminal under the strongly hooked and more or less flattened tip of the style; leaf rachis not winged.

15. CORYNELLA.

Stigma terminal at the end of the curved but scarcely hooked style.

Rachis of the leaves not winged; keel broad, strongly arcuate, not exceeding the banner in length, the blades nearly semi-circular.

14. SABINEA.

Rachis of the leaves distinctly wing-margined; keel narrower, less arcuate, longer than the banner.

16. NOTODON.

Calyx 2-lipped; banner obovate; style bent at the base, thereafter nearly straight; petals subequal; rachis winged.

17. BEMBICIDIUM.

#### 14. *Sabinea* DC. Ann. Sci. Nat. 4: 92. 1825

Trees or shrubs. The leaves are abruptly pinnate, with stipules and sometimes with minute stipels. The flowers are purplish, in small fascicles or short racemes in the axils of old leaves, appearing with or before the new leaves; bracts small, bractlets none. The calyx is open-campanulate or turbinate, truncate, with 5 minute lobes. The banner is short-clawed, spreading or reflexed; the wings short-clawed, with oblong or oblanceolate, slightly falcate blades and with a basal auricle; the keel-petals clawed, rounded at the apex, with obliquely oblanceolate or obovate, nearly semi-circular blades. Stamens 10, diadelphous, the free portion of at least the 5 lower filaments as long as the sheath, the filaments subequal or the lower 5 much longer than the upper. The style is arcuate, incurved. The pod is stipitate, flat, acute at each end, 2-valved, with neither partitions nor constrictions between the seeds. The seed are compressed, ovate.

ILLUSTRATION: Plate XXXIII, *N. Sabinea florida* (Vahl) DC.: 1. flowering branch, 2. leaf,  $\times \frac{2}{3}$ ; 3. calyx, 4. stamens, 5. pistil, 6. banner,

7. wing, 8. keel-petal,  $\times 1$ . *S. punica* Urb.: 9. stamens,  $\times 1$ ; 10. young pod, 11. cross section of the same,  $\times \frac{2}{3}$ .

The genus was based on *Robinia florida* Vahl. and consists of 3 species, natives of Porto Rico and some of the Lesser Antilles. De Candolle also included "*R. dubia* Lam." (Poir.), which, however, does not agree with his diagnosis and apparently belongs to *Corynella*.

15. *Corynella* DC. Ann. Sci. Nat. 4: 93. 1825

Low shrubs. The leaves are abruptly pinnate, with subulate spinescent erect stipules and coriaceous leaflets. The flowers are purple, fascicled on short axillary branches, appearing before the leaves. The calyx is campanulate, with short lobes. The banner is broadly obovate or suborbicular, short-clawed; the wings lanceolate or oblong, with rather conspicuous basal auricles and strongly curved claws; the keel-petals are longer than the banner, clawed, their blades strongly lunate, with obtuse to acuminate tips and small basal auricles. The stamens are diadelphous, the staminal sheath equally broad throughout, the filaments subequal. The style is glabrous, bent at the base, strongly hooked and dilated at the end, the stigma subterminal, under the tip. The pod is linear, slightly stipitate, many-seeded, not torulose.

ILLUSTRATION: Plate XXXIII, O. *Corynella dubia* (Poir.) Urb.: 1. flowering branch, 2. leaves,  $\times \frac{2}{3}$ ; 3. calyx, 4. stamens and pistil, 5. banner, 6. wing, 7. keel-petal,  $\times 2$ ; 8. pod, 9. cross section of the same,  $\times 1$ ; 10. seed,  $\times 2$ .

The genus was established on *Robinia polyantha* Sw. and *Corynella paucifolia* DC., of which the former is taken as the type. It is evidently the same as *Robinia dubia* Poir. and becomes *Corynella dubia* (Poir.) Urb.

Synonym:

*Corynites* Spreng. Syst. 4, Cur. Post. 263, 286. 1827. This was merely a change of name, and based on the same species.

The genus is closely related to the preceding, characterized by the flattened, strongly incurved style, clavate at the apex, and bearing the small stigma under the incurved tip. In *C. dubia* the keel-petals are acuminate and united to the tip; in *C. paucifolia* they are acute or obtuse with free tips.

16. *Notodon* Urb. Symb. Ant. 1: 324. 1899

Shrubs, with or without infra-petiole curved prickles. The leaves are abruptly pinnate; the stipules lanceolate, with a subulate stiff apex, deciduous; the petiole and rachis more or less winged, the wings discontinuous at the insertion of the leaflets, the rachis produced into a short point, the leaflets entire, coriaceous, veinless; the stipules minute. The flowers are borne in small fascicles in the axils of old leaves, appearing before the new ones. The calyx is short-campanulate, remotely and minutely 5-lobed, the upper two lobes close together; the banner is suborbicular, reflexed with a very short claw; the wings oblong, straight, with a curved claw; the keel-petals united by their upper half, lunate, with the base truncate on one side, obtuse, much longer than the banner. The stamens

are diadelphous, the upper filament free, the rest united into a linear sheath, nearly equal in length. The ovary is short-stipitate, linear, several-ovuled, the style arcuate, glabrous; the stigma minute, terminal. The pod is linear-oblongate, acute at both ends.

ILLUSTRATION: Plate XXXIII, *P. Notodon gracilis* (Griseb.) Urb.: 1. flowering branch,  $\times 1$ ; 2. calyx, 3. stamens, 4. pistil, 5. banner, 6. wing, 7. keel-petal,  $\times 2$ ; 8. fruiting branch,  $\times 1$ .

The type of the genus is *Fagara gracilis* Griseb., which was originally described from specimens in leaves only. It was mistaken for a *Fagara* (*Xanthoxylum*), on account of its winged leaf rachis and short spines. Three more species have been discovered lately. They are spineless, but otherwise closely resemble the original one. All are natives of Cuba.

17. *Bembicidium* Rydberg, Mem. Torrey Bot. Club 16: 68. 1920

Low unarmed shrubs. The leaves abruptly pinnate; the stipules lanceolate, persistent; the petiole and rachis broadly winged, the wings discontinuous at the nodes, the rachis slightly produced above the uppermost leaflets; the leaflets entire, coriaceous, without veins, the midrib prominent beneath, obsolete above; the stipels obsolete. The flowers are solitary in the axils. The calyx is turbinate, tapering at the base, as broad as long, with two broad, subequal, acute lips. The corolla is purplish, with subequal petals; the banner is obovate, slightly retuse, gradually tapering into the short, broad claw; the wing and keel-petals are equal in length and shape, the blades obliquely oblanceolate, rounded at the apex, slightly auricled at the base, the claws short, straight; the blades of the keel-petals united at the middle only. The ovary is slightly stipitate, linear, many-ovuled; the style glabrous, bent inward at the base, slightly arcuate, not hooked at the apex, the stigma minute, terminal. The fruit is unknown.

ILLUSTRATION: Plate XXXIII, *Q. Bembicidium cubense* Rydb.,  $\times 1$ : 1. calyx, 2. pistil, 3. banner, 4. wing, 5. keel-petal,  $\times 2$ .

This is a monotypic genus, based on *Bembicidium cubense* Rydb., in habit resembling the species of *Notodon*, having like them a winged leaf rachis, but the banner is not shorter than the other petals and the calyx is 2-lipped.

SUBTRIBE 8. ROBINIANAE

Trees or shrubs, or rarely herbs, suffrutescent only at the base. The leaves are odd-pinnate, or, in some species of *Coursetia* and occasionally in *Olneya*, abruptly pinnate, with petioluled leaflets and often with small stipels, or the rachis with more or less distinct nodes. The flowers are borne in axillary racemes, usually with caducous bracts, without bractlets. The calyx has mostly a campanulate tube and 5 lobes. The corolla usually with subequal petals, except in *Sauvallella* and *Poitea*. The stamens are diadelphous or monadelphous; the anthers subequal. The ovary is many-ovuled, stipitate or sessile; the style inflexed or incurved; the stigma terminal, minute. The pod is linear, flat, 2-valved, several-seeded. The seeds are without strophiole, usually compressed.

The subtribe consists of the following twelve genera, all natives of America. To me it seems that *Homalobus* and *Kentrophyta*, segregates



of *Astragalus*, should also be included herein, as there are no characters either in the flower or the fruit by which they could be excluded. They are, however, herbaceous, but so are also *Sphinctospermum* and some species of *Benthamantha*. On the other hand, there are also many shrubby species which have been included in the subtribe *Astragalanae*.

Style more or less hairy.

Style, at least the upper part, hairy all around, stipular spines often present.

Pod flat, wing-margined or ridged on the seed-bearing suture, banner without callosities; plant not canescent.

18. *ROBINIA*.

Pod turgid, neither wing-margined nor ridged; banner with two callosities; plant canescent.

19. *OLNEYA*.

Style hairy on the upper side only; spines absent.

Style long-hairy, bent, but not spirally incurved.

Pod not strongly impressed between the orbicular seeds.

20. *COURSETIA*.

Pod strongly impressed between the seeds, which are nearly rectangular in outline.

Seed not constricted at the middle; plant suffruticose with odd-pinnate leaves.

28. *BENTHAMANTHA*.

Seeds constricted at the middle; annual herbs with simple leaves.

29. *SPHINCTOSPERMUM*.

Style short-hairy, spirally incurved.

21. *LENNEA*.

Style glabrous.

Stamens more or less monadelphous; the upper filament free at the base, united with the sheath at least at the middle.

Calyx-lobes 5, short, distinct; stipules absent.

22. *WILLARDIA*.

Calyx-lobes apparently 4, *i.e.*, the upper two united to or nearly to the apex; stipules present, filiform.

23. *HESPEROTHAMNUS*.

Stamens diadelphous.

Keel-petals united up to the apex, more or less arcuate; staminal sheath not dilated at the apex.

Stigma pubescent; pod with woody-corky masses between the seeds.

24. *HEBESTIGMA*.

Stigma not pubescent; pod not corky between the seeds.

Petals subequal; keel-petals obtuse.

25. *GLIRICIDIA*.

Petals very unequal, the banner the shortest, the keel-petals the longest, acute.

26. *SAUVALLELLA*.

Keel-petals narrow, free at the tip; banner shorter than the keel-petals; staminal sheath dilated at the apex.

27. *POITEA*.

# 18. *Robinia* L. Sp. Pl. 722. 1753

Trees or shrubs. The leaves are odd-pinnate, with setaceous or spinose stipules, and small stipels. The flowers are borne in axillary racemes, with caducous bracts. The calyx is more or less 2-lipped, the upper lip with two broad, short teeth united to near the tip, the lower one more deeply 3-lobed. Corolla is white or purple; the banner with a broad blade and short claw; the wings are free with obliquely oblong or obovate blades and with a basal auricle, the keel-petals obtuse, incurved, with obliquely obovate blades, auricled at the base, and united from the middle to the tip. The stamens are more or less monadelphous, the upper filament free at the base, more or less united with the sheath at the middle. The ovary is more or



less stipitate, many-ovuled, the style short-inflexed, short-hairy towards the apex; the stigma terminal, minute. The pod is linear, flat, 2-valved, continuous within, narrowly winged or merely ridged along the upper suture. The seeds are oblong, oblique, without strophiole, but with a rather slender funicle.

ILLUSTRATION: Plate XXXIV, *R. Robinia Pseudo-Acacia* L.,  $\times \frac{1}{3}$ : 1. calyx, 2. stamens, 3. pistil,  $\times 2$ ; 4. banner, 5. wing, 6. keel-petal,  $\times 1$ ; 8. pod, 7. cross section of the same,  $\times \frac{2}{3}$ .

The type of the genus is *Robinia Pseudo-Acacia* L. The species number about 20, natives of North America.

Synonym:

*Pseudo-Acacia* (Tourn.) Medic. Vorl. Churpf. Phys. Ges. 2: 364. 1797.

This was based on the same type.

Traubert distinguished *Robinia* from the other genera of the subtribe by the fruit's being winged on the upper suture; in fact, it is only in *R. Pseudo-Acacia* and maybe *R. Pringlei* that it can be called narrowly winged; in the other species it is merely ridged or with an acute margin.

19. *Olneya* A. Gray, Mem. Amer. Acad. II, 5: 328. 1855

Canescent spinose trees. The leaves are abruptly pinnate with obsolete stipules and no stipels. The calyx is campanulate, 5-lobed; the upper two lobes are united to near the apex. The banner is short-clawed, its blade orbicular, emarginate, reflexed, with two callosities at the base; the wings are obliquely obovate, falcate, with a small basal auricle, short-clawed; the keel-petals are short-clawed, their blades broadly lunate, almost semi-circular, with a broad, rounded basal auricle. The stamens diadelphous, nearly of equal length, the sheath cylindric, curved; the anthers uniform. The ovary is short-stipitate, several-ovuled, the style arcuate, incurved, pubescent above, the stigma capitate, terminal. The pod rather turgid, 2-valved, torulose, puberulent and glandular-hispid. The seeds are rounded-ellipsoid, scarcely compressed.

ILLUSTRATION: Plate XXXIV, *S. Olneya Tesota* A. Gray,  $\times \frac{2}{3}$ : 1. calyx, 2. stamens, 3. pistil,  $\times 2\frac{1}{2}$ ; 4. banner, 5. wing, 6. keel-petal,  $\times 2$ ; 7. pod, 8. cross section of the same,  $\times 1$ .

The genus is monotypic, based on *Olneya Tesota* A. Gray, a native of Arizona, Southern California, Sonora, and Lower California.

Synonym:

*Tesota* C. Mueller; Walp. Ann. 379. 1857. The type species is not designated but evidently the same.

The genus is intermediate between *Robinia* and *Coursetia*, characterized by the terete and torulose pod, and the two callosities at the base of the blade of the banner.

20. *Coursetia* DC. Ann. Sci. Nat. 4: 92. 1824

Shrubs or trees. The leaves are abruptly pinnate or odd-pinnate, with many entire petiolulate leaflets and narrow subulate persistent and somewhat spinescent stipules. The flowers are borne in axillary racemes, soli-

tary in the axils of small deciduous bracts. The calyx-tube is campanulate or turbinate, about as broad as high; the lobes subequal in length, but the upper two often united higher up. The corolla is white, ochroleucous, reddish or purplish; the petals subequal or the wings shorter. The banner is suborbicular with a short claw, sometimes with a pair of callosities. The wings are short-clawed, the blades obliquely oblanceolate or oblong, with a basal auricle; the keel-petals have somewhat longer claws, the blades are broader, with a small basal auricle, obtuse to acuminate. The stamens are diadelphous or monadelphous, the upper filament bent near the base, the sheath somewhat dilated below. The style is strongly inflexed at the base, then straight, hairy above on the upper side. The pod is linear, 2-valved, compressed, without partitions, usually constricted around the seed and more or less torulose, sessile or short-stipitate, but the lower portion often narrower and without seeds. The seeds are orbicular, without strophiole, compressed.

ILLUSTRATION: Plate XXXIV, *T. Coursetia microphylla*,  $\times \frac{2}{3}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 2$ ; 7. pod, 8. cross-section of the same,  $\times 1$ . *C. madrensis*: 9. banner, 10. wing, 11. keel-petal,  $\times \frac{2}{3}$ .

The type is *Coursetia tomentosa* DC., based on *Astragalus fruticosus* Cav., a Peruvian species. DeCandolle also included *Aeschynomene virgata* Cav., but this is evidently a species of Daubentonia.

Synonym:

Humboldtiella Harms, Repert. Sp. Nov. 19: 12. 1923. The type was *H. ferruginea* (H.B.K.) Harms, based on *Robinia ferruginea* H.B.K.

The genus, as usually constituted, is rather complex. It consists of perhaps a score of species, natives of Mexico, Central and South America, two species extending into southwestern United States. The species are quite varying in habit and it is possible that the genus should be divided into several genera. In some species, as for instance in the type, the leaves are abruptly pinnate and usually without stipels. To this group belong *C. glandulosa* A. Gray and three other species from Mexico and the United States, and *C. arborea* from the Lesser Antilles, northern South America, and Panama. In other species the leaves are odd-pinnate and mostly with stipels. To this group belong 3 Mexican and several South American species; *C. madrensis* has also odd-pinnate leaves, but differs from the rest in having two callosities on the banner and acuminate keel-petals. The species with abruptly pinnate leaves connect the subtribe Robinianae with the Corynellanae, but they are evidently more related to Robinia and Olneya than to Corynella or Sabinea, as the flowers are borne in axillary racemes appearing after the leaves, and the style is pubescent.

Humboldtiella is distinguished from Coursetia according to Harms by its short and broad calyx-lobes and by the vexillar filament, which is united with the staminal sheath at the middle. Harms evidently regarded *C. glandulosa* and its allies as typical Coursetia. These characters do not hold when all the species are considered. He also regarded *C. arborea*

Griseb. as synonymous with *Robinia ferruginea*. In the former the fruit is glabrous or nearly so and the flowers appear with the leaves. According to him the type of the latter is leafless and the ovary densely tomentose.

21. *Lennea* Klotzsch; Link, Klotzsch, & Otto, Ic. Pl. Rav. Hort. Berol. 2: 65. 1844

Trees or shrubs, with odd-pinnate leaves and subulate or setaceous deciduous stipules and stipels. The flowers are borne in axillary racemes, with subulate or setaceous bracts. The calyx is campanulate, with 5 short teeth. The corolla has 5 subequal petals; the banner is reflexed, the blade orbicular, the claw short; the wings are short-clawed; the blades obliquely oblong, with a basal auricle; the keel-petals are somewhat lunate, obtuse, united above, with a rounded basal auricle. The stamens are 10, monadelphous, the upper filament free and somewhat kneed at the base, then united with the sheath to above the middle, and then again free; the anthers uniform. The ovary is short-stipitate, many-ovuled; the style short-pubescent along the upper margin, strongly spirally curved towards the apex; the stigma terminal. The pod is linear, slightly stipitate, many-seeded, without cross partitions. The seeds are lenticular.

ILLUSTRATION: Plate XXXIV, U. *Lennea robinoides* Klotzsch,  $\times \frac{2}{3}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 2$ ; 7. young pod,  $\times 1$ . *L. brunnescens* Standl.: 8. cross section of the pod, 9. seed,  $\times \frac{2}{3}$ .

The genus was established on *Lennea robinoides* Klotzsch and consists of three species, two of Mexico and one of Panama.

22. *Willardia* Rose, Contr. U. S. Nat. Herb. 1: 97. 1890

Small trees or shrubs. The leaves are odd-pinnate, with petiolulate entire leaflets, deciduous stipules, and no stipels. The flowers are borne in axillary racemes; bractlets are absent. The calyx is short-campanulate, fully as broad as long, truncate, with fine, minute, equal teeth. The corolla is lilac, white, or ochroleucous, the banner and keel subequal, the wings somewhat shorter; the banner is suborbicular, retuse at the apex, reflexed, short-clawed; the wings are short-clawed, the oblong-falcate blades apparently without a basal auricle; the keel-petals are lunate, equaling the calyx. The stamens are monadelphous; the upper filament is free at the base, otherwise united with the rest into a closed sheath; the anthers are uniform. The ovary is subsessile, several-ovuled; the style incurved, glabrous; the stigma small, capitate. The pod is flat, oblong-lanceolate, acute at each end, without partitions, 2-valved. The seeds are reniform, strongly compressed.

ILLUSTRATION: Plate XXXV, V. *Willardia eriophylla* (Benth.) Standley,  $\times \frac{2}{3}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 1$ ; 7. pod,  $\times \frac{2}{3}$ ; 8. cross section of the same,  $\times 1$ .

The genus was established on *Coursetia mexicana* S. Wats. and consists of 3 Mexican species. It is intermediate between *Coursetia*, *Robinia*, and *Olneya* on one hand and *Gliricidia* and *Hebestigma* on the other, differing from the former group in the glabrous style and from the latter in the monadelphous stamens.

23. *Hesperothamnus* Brand. Univ. Calif. Publ. Bot. 6: 499. 1919

Shrubs or small trees. The leaves are odd-pinnate, with petioluled entire leaflets, deciduous stipules, and filiform stipels. The flowers are borne in axillary racemes or raceme-like panicles, *i.e.*, the flowers several, fascicled on short branches at each node of the peduncle, with filiform bracts and no bractlets. The petals are subequal, the claw is slender, nearly half as long as the blade; the blade of the banner rounded-obovate, erect, those of the wings oblong, falcate, rounded at the apex, truncate on the upper margin at the base, those of the keel-petals strongly lunate, their upper half united, the apex obtuse, and the base with a rounded auricle. The stamens are monadelphous, the upper filament free at the base, adnate to the staminal sheath at the middle; the anthers uniform. The ovary is subsessile, several-ovuled; the style abruptly bent at the base, glabrous; the stigma minute, capitate. The pod flat, linear or linear-oblong, acute at each end, 2-valved, without partitions. The seeds are suborbicular, compressed.

ILLUSTRATION: Plate XXXV, *W. Hesperothamnus littoralis* Brand.,  $\times \frac{1}{2}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 2$ ; 7. pod, 8. cross section of the same,  $\times 1$ .

The genus was based on *Lonchocarpus littoralis* Brand.

Synonym:

*Selerothamnus* Harms, Rep. Spec. Nov. 17: 325. 1921. It is based on three species, of which *S. pentaphyllus* Harms is the type.

Harms in describing his new genus suggested that *Lonchocarpus littoralis* Brand. might belong to *Selerothamnus*, but he had overlooked the fact that this had already been made the base of a new genus, *Hesperothamnus*, by Brandegee. Harms thought that the genus is related to *Galactia*. He admitted that the fruit was unknown to him. It is evident that the genus is closely related to *Willardia*, differing mainly in the calyx, which has unequal lobes in which the upper two are wholly or nearly wholly united. The genus consists of 4 or 5 Mexican species.

24. *Hebestigma* Urban, Symb. Ant. 2: 289. 1900

Trees, with subopposite or alternate, odd-pinnate leaves, apparently without stipules and stipels. The flowers are borne in racemes, theoretically axillary (but the subtending leaves often not developed), appearing before and below the leaves on young shoots. The calyx is short, obliquely turbinate, 5-toothed, the lobes short and broad. The banner is orbicular, reflexed, without callosities, short-clawed; the wings are obliquely obovate, clawed, with a prominent basal auricle; the keel-petals are obovate-falcate, obtuse, united at the apex, with a rounded basal auricle. The stamens are diadelphous; the staminal sheath cylindric; the anthers uniform. The ovary is stipitate, 5-9-ovuled; the style glabrous, inflexed at the base at nearly a right angle, subulate; the stigma terminal, villous. The pod is subsessile, woody, linear, flat, 2-valved, the exocarp leathery, the endocarp woody, with woody-corky masses between the seeds. The seeds are compressed, suborbicular-obovate, suberect.

ILLUSTRATION: Plate XXXV, Y. *Hebestigma cubense* (H. B. K.) Urban,  $\times \frac{1}{2}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 1$ ; 7. pod, 8. cross section of the same,  $\times \frac{1}{2}$ .

The genus is monotypic, based on *Robinia* (?) *cubensis* H. B. K. It is most closely related to *Gliricidia*, differing in the hairy stigma and woody pod, with woody cross partitions between the seeds. The tree is confined to the island of Cuba.

25. *Gliricidia* H. B. K. Nov. Gen. et Sp. 6: 393. 1823—Stend. Nomencl., ed. 2, 1: 688. 1841

Trees or shrubs, with odd-pinnate leaves and small stipules but no stipels. The flowers are borne in axillary racemes, often appearing before the leaves; the bracts are small, deciduous; the bractlets none. The calyx is short-campanulate, as broad as long, truncate, the teeth 5, broad and short, or obsolete. The banner orbicular, reflexed, often with two callosities at the base of the blade, short-clawed; the wings are oblong-ob lanceolate, erect, free, with a basal auricle, short-clawed; the keel-petals are strongly arcuate above the middle, obtuse, united at the end, tapering at the base. The stamens are diadelphous; the sheath is cylindric, not dilated at either end; the anthers uniform. Ovary stipitate, 7-12-ovuled; the style glabrous, inflexed at nearly a right angle; the stigma capitate, papillose, not pubescent. The pod is short-stipitate, linear, compressed, 2-valved. The seeds are rounded, compressed.

ILLUSTRATION: Plate XXXV, Z. *Gliricidia sepium* (Jacq.) Stend.,  $\times \frac{1}{4}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 1$ ; 7. pod, 8. cross section of the same, 9. developed leaf,  $\times \frac{1}{2}$ .

The genus was based on *Robinia* (?) *maculata* H. B. K. and *R. sepium* Jacq., of which the former may be regarded as the type. As Kunth in "Nova Genera et Species" only suggests that the two species may constitute a genus, proposing tentatively the name without naming the species, the real publication may be regarded as dating from 1841, when the combinations were made by Stendel.

Synonym:

Hybosema Harms, Repert. Sp. Nov. 19: 67. 1923.

The type of this genus was *H. Ehrenbergii* (Schlecht.) Harms, based on *Robinia Ehrenbergii* Schlecht., which, according to Harms, may be the same as *Gliricidia guatemalensis*.

The genus consists of at least 6 species, natives of Mexico and Central America; only *G. sepium* is also found in the West Indies and northern South America. It may be divided into two groups of 3 species each: the first, of which *G. sepium* is representative, with large flowers, 15 mm. long or more, large leaflets, and with the three lower calyx-lobes distant; and the second with *G. guatemalensis* as representative, with small flowers less than 12 mm. long, small leaflets, and the lower calyx-lobes close together. This group constitutes the genus *Hybosema* Harms. The somewhat 2-lipped calyx, however, seems to me a rather poor generic character.

26. *Sauvallella* Rydberg, gen. nov.

Shrubs, with odd-pinnate leaves, subulate free stipules, and minute lanceolate stipels. The flowers are borne in axillary racemes, with small bracts and no bractlets. The calyx is short-turbinate, broader than high, the teeth are minute, the lower three triangular-subulate, the upper two minute, close together, obtuse. The banner suborbicular, short-clawed, with somewhat spreading sides, shorter than the wings; the wings obliquely obovate, short-clawed, with a prominent basal auricle; the keel-petals lunate, acute, united to the apex, longer than the other petals. The stamens are diadelphous; the sheath is cylindric, not dilated above. The ovary is many-seeded, glabrous; the style glabrous, slightly curved at the base; the stigma terminal. The pod is flat, linear, stipitate, many-seeded.

ILLUSTRATION: Plate XXXVI, A. *Sauvallella immarginata* (Wright) Rydb.,  $\times \frac{2}{3}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 2$ ; 7. young pod,  $\times 1$ .

The type species is *Sauvallella immarginata* (Wright) Rydb., based on *Corynella immarginata* Wright; Sauv. Fl. Cub. 26. 1868.

This species must be excluded from *Corynella*, as the leaves are odd-pinnate instead of abruptly pinnate, the flowers in short axillary racemes, the calyx-lobes short and subequal, the style nearly straight and terete, neither strongly incurved nor flattened, and the stigma terminal, not situated under the tip of the style. The new genus is intermediate between *Gliricidia* and *Poitea*. In the former, the style and the free portions of the filaments are strongly bent upwards; in *Sauvallella* they are nearly straight; in *Gliricidia*, the petals are subequal or the banner longer than the keel and its borders reflexed; in *Sauvallella* the keel-petals are much longer than the banner, which is conduplicate around the other petals. *Sauvallella* differs from *Poitea* in the broader keel-petals, which are united to the tip, and in the staminal sheath which is not dilated towards the tip.

The genus is dedicated to Francisco A. Sauvalle, who published a revised catalogue of the plants collected in Cuba by Charles Wright.

27. *Poitea* Vent. Choix 36. 1800.

Shrubs with distichous odd-pinnate leaves, without stipels, the stipules subulate from a broader base, united with each other, inside the petioles. The flowers are borne in axillary racemes, with small bracts and no bractlets. The calyx is cylindro-turbinate, acute at the base, more or less distinctly articulate, 5-lobed, the lobes short and the upper two close together. The corolla is reddish-purple to white; the banner obovate, usually retuse, narrowed at the base but scarcely clawed, enclosing the bases of the other petals, not reflexed; the wings are narrow, straight, linear or linear-oblancoate; the keel-petals narrow, straight, longer than the banner, oblanceolate, obtuse, only slightly united, but with free tips. The stamens are diadelphous, often exserted; the sheath long and dilated above. The ovary is stipitate, many-ovuled; the style straight or slightly curved upward, glabrous; the stigma minute, terminal. The pod is flat, linear, stipitate, 2-valved. Seeds compressed, orbicular or obovate.



ILLUSTRATION: Plate XXXVI, *B. Poitea galegioides* Vent.,  $\times \frac{1}{2}$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 1$ ; 7. pod,  $\times 1$ .

The genus was established on *Poitea galegioides* Vent.

Synonym:

Wilmorinia DC. Prod. 2: 239. 1825. Type, *W. multiflora* based on *Clitoria multiflora* Sw.

The genus consists of 5 species, all natives of Hispaniola.

28. *Benthamantha* Alef. Bonplandia 10: 264. 1862

Suffrutescent perennials. The leaves are odd-pinnate, with stipules and stipels. The flowers are borne in axillary racemes, with subulate or setaceous, caducous bracts. The calyx has a companulate tube and 5 subequal lobes, with setaceous or subulate acuminations. The corolla is yellowish or white, the petals subequal; the banner is clawed, the blade orbicular or reniform, with reflexed margins; the wings are clawed, with oblong blades, free; the keel-petals broadly obliquely obovate, acute or acuminate, united to the apex. The stamens are diadelphous. The ovary is sessile, many-ovuled; the style rigid, inflexed, longitudinally bearded above; the stigma capitate. The pod is linear, flat, 2-valved, conspicuously constricted between the seeds externally, internally with cross partitions. Seeds subrectangular or subquadrate, without strophiole.

ILLUSTRATION: Plate XXXVI, *C. Benthamantha caribaea* (Jacq.) Kuntze,  $\times 1$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal,  $\times 2$ ; 7. pod,  $\times 1$ ; 8. cross section of the same, 9. seed,  $\times 2$ .

The genus was based on the genus *Cracca* Benth., which was untenable on account of the older *Cracca* L. The type is therefore the same as that of *Cracca* Benth.

Synonyms:

*Cracca* Benth.: Benth. & Oerst. Vidensk. Medd. 1853: 8. 1853; not L. 1753. This genus was established on 6 species, five of which had been included in *Tephrosia*. The first of these was *Cracca glandulifera* Benth., which may be regarded as the type.

*Brittonamra* Kuntze, Rev. Gen. 164. 1891. This was also a substitute for *Cracca* Benth., the type therefore being the same.

The genus consists of 20 species, natives of Mexico and Central America. Three of these extend into the southwestern United States, one into the West Indies, and a few into South America, where there are also half a dozen additional species. In general habit the species resemble a good deal certain ones of *Cracca* L. (*Tephrosia* Pers.), but the flower-clusters are strictly axillary and the pods are furnished with false cross partitions between the seeds. In this last character this genus and the next differ from the rest of the Robinianae. On account of the latter character and the more or less herbaceous habit, the two genera constitute a group by themselves, not very closely related to the rest.



29. *Sphinctospermum* Rose, Contr. U. S. Nat. Herb. 10: 107. 1906

Annual herbs, with unifoliolate linear leaves. The flowers are single or rarely paired in the axils, short-pediceled, with minute bracts and no bractlets. The calyx is turbinate, with 5 subequal lobes. The corolla is pinkish; the banner rounded, obcordate, with a very short claw; the wing-petals are short-clawed, the blade obliquely oblong with an acute basal auricle; the keel-petals strongly lunate, united above, almost semi-orbicular, with a prominent basal auricle. The stamens are 10, diadelphous, the upper filament free, the other nine united less than half their length, the alternate ones shorter; the anthers uniform. The ovary is sessile; the style slender, hairy near the tip. The pod is linear, flat, 2-valved, transversely septate between the seeds. The seeds are prismatic, constricted at the middle, roughened.

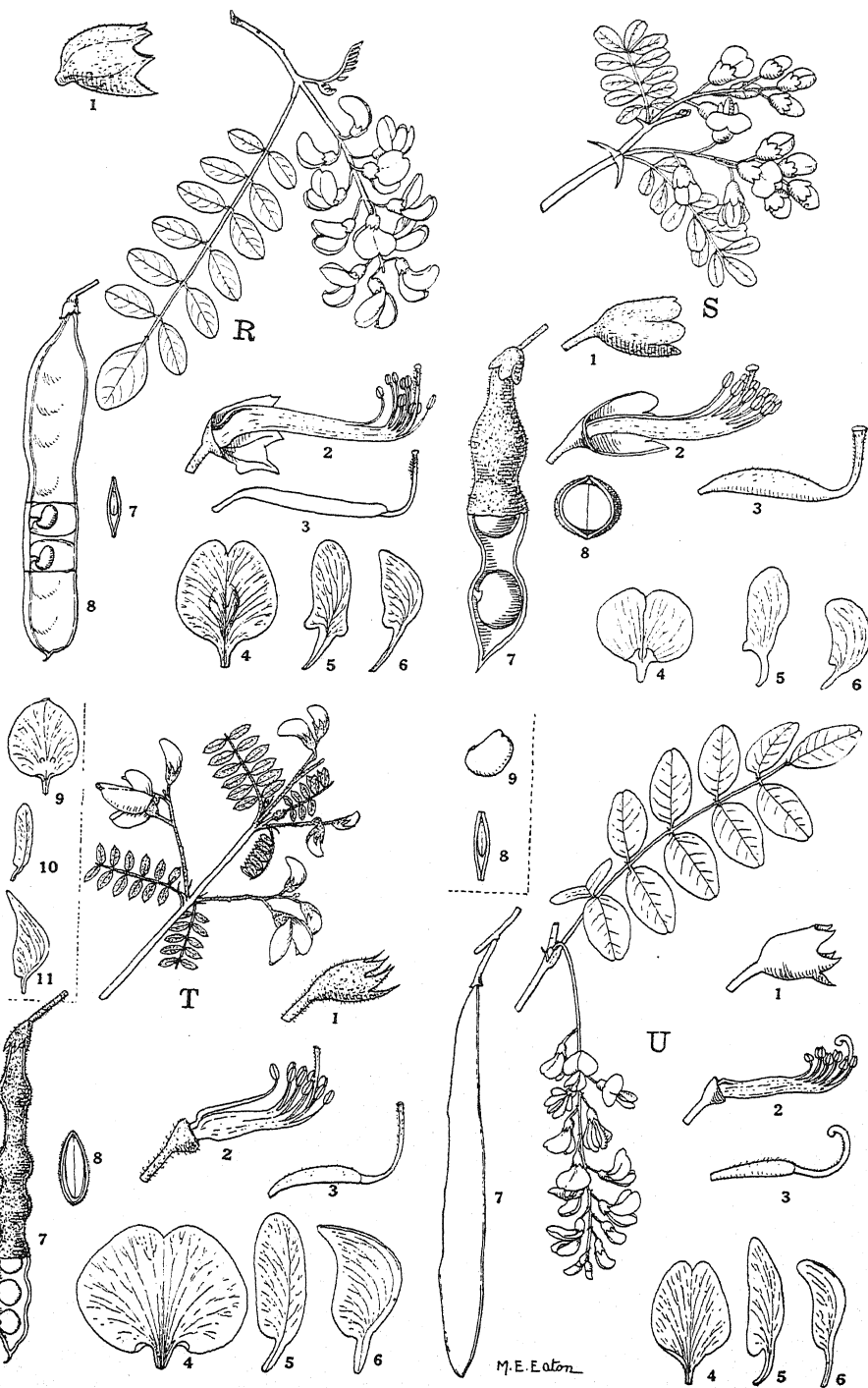
ILLUSTRATION: Plate XXXVI, *D. Sphinctospermum constrictum* (S. Wats.) Rose,  $\times 1$ : 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petals, 7. pod, 8. cross section of the same,  $\times 2$ ; 9. seed,  $\times 2\frac{1}{2}$ .

The genus is monotypic, based on *Tephrosia constricta* S. Wats. It is evidently closely related to *Benthamantha*, though hitherto associated with *Cracca* L.



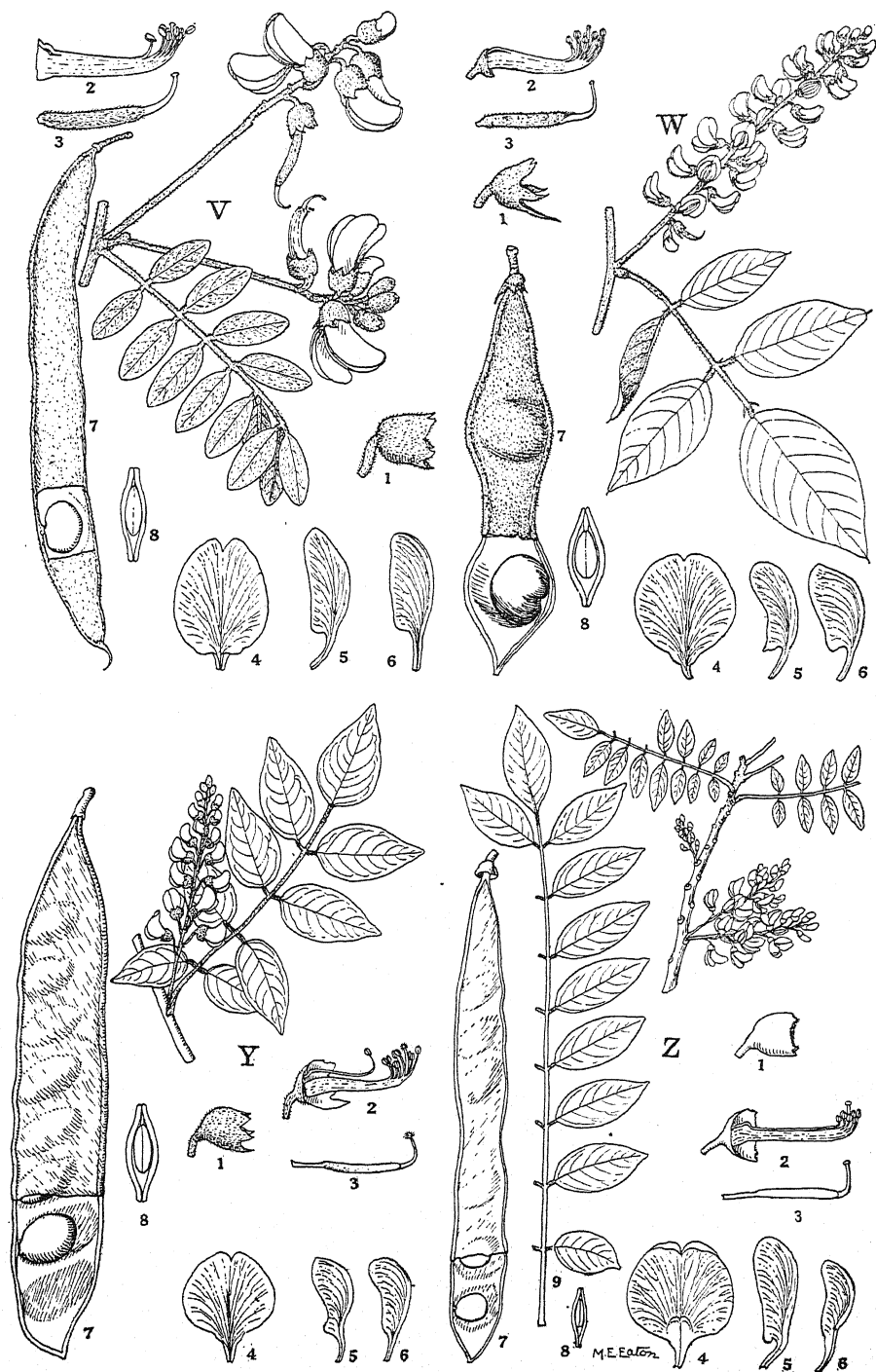
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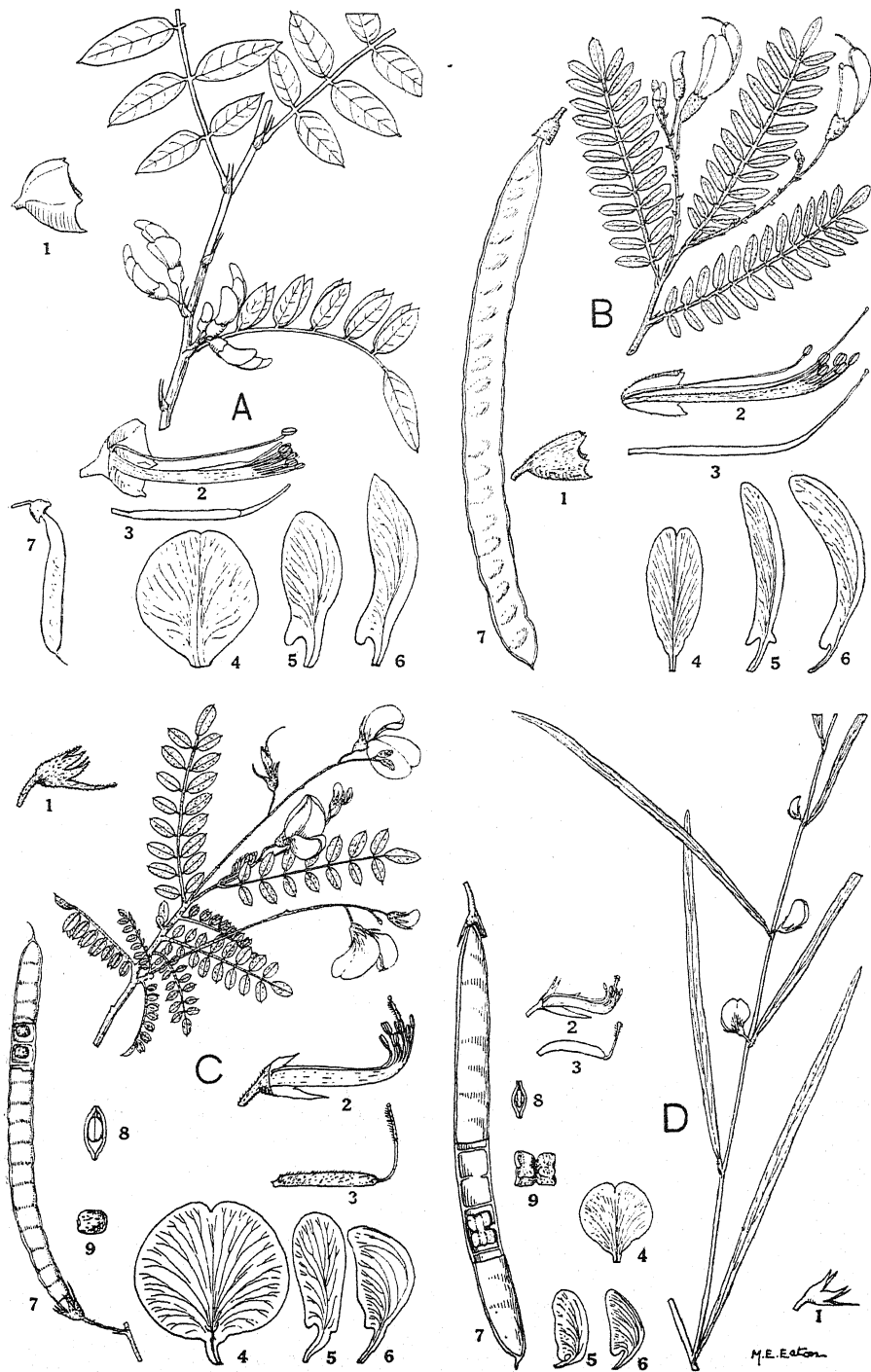




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RYDBERG: NORTH AMERICAN FABACEAE



## PHYSIOLOGIC RACES OF OAT SMUTS<sup>1</sup>

GEORGE M. REED

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Physiologic specialization is of very general occurrence among the parasitic fungi. In 1918 the writer (4) summarized the information then available, but since that time a large number of additional investigations have been carried out, all of which greatly extend our knowledge along the line of the specialization of parasites to particular hosts.

For the most part the smuts have been a neglected group for this type of investigation. While various suggestions have been made regarding the occurrence of specialization in the smuts, it remained for Zillig (9) first to establish experimentally the fact that *Ustilago violacea* (Pers.) Fuck. consists of a number of different races definitely limited to specific hosts. Until recently no specific information regarding a similar specialization among the cereal smuts has been available, although certain facts have pointed to the possible existence of physiologic races. Faris (1), however, has demonstrated the occurrence of specialization in the covered smut of barley, *Ustilago hordei* (Pers.) K. & S. Distinct races can be recognized by their capacity for infecting particular varieties of barley.

The writer has long been interested in the problem of the varietal resistance of oats and other cereals to their respective smuts. In 1920 a paper (5) embodying his results on the behavior of oat varieties to *Ustilago avenae* (Pers.) Jens. and *U. levis* (K. & S.) Magn. was published. Numerous varieties and strains were used which belonged to the following species of *Avena*: *A. brevis* Roth, *A. fatua* L., *A. nuda* L., *A. sativa* L., *A. sativa orientalis* L., *A. sterilis* L., and *A. strigosa* Schreb. All the strains of the two species of *Avena brevis* and *A. strigosa* which were grown proved to be entirely free from infection by both smuts. The strains and varieties of *A. fatua*, *A. nuda*, and *A. sativa orientalis* were moderately or extremely susceptible to both smuts. Practically all of the varieties of *A. sativa* also were quite susceptible. However, a great deal of variation occurred, and some varieties seemed to be quite resistant. Two strains of the variety Black Mesdag consistently gave negative results. The strains and varieties of *A. sterilis* proved to be only slightly susceptible to both smuts. Certain cultivated varieties, as Burt, Fulghum, and Red Rustproof, appeared to be particularly resistant. For the most part the varieties responded similarly

<sup>1</sup> Brooklyn Botanic Garden Contributions no. 38. Published, at the expense of the Brooklyn Botanic Garden, out of the order determined by the date of receipt of the manuscript.

to both loose and covered smut, although some differences in their behavior were observed.

Heald (2) has reported results with *Ustilago levis* on nineteen varieties of oats. In general his data are in harmony with those obtained by the writer. The only important exception is found in the variety Kherson, which gave negative results. This variety in the writer's experiments was among the susceptible ones.

Vavilov (8), in Russia, also has reported his observations on the behavior of a large number of varieties of oats with reference to *Ustilago avenae*. Most of the common species of *Avena* were represented by several strains and varieties. His results coincide quite closely with those obtained by the writer. Vavilov found that strains and varieties of *Avena brevis*, *A. strigosa*, and *A. sterilis* were resistant. On the other hand, the varieties of *A. fatua*, *A. nuda*, *A. sativa*, and *A. sativa orientalis* were susceptible. One immune variety of *A. sativa* was noted. This was Mesdag, which appears to be identical with Black Mesdag which proved free from smut in the writer's experiments. Vavilov also recorded that *A. nuda* L. var. *bi-aristata* was somewhat resistant and that one strain of *A. strigosa* proved to be susceptible. This strain, however, appears to be genetically different from others of this species, since it can be readily crossed with varieties of *A. sativa*.

Sampson and Davies (7) have recorded the occurrence of *Ustilago avenae* on thirty-one varieties of oats in the experimental plats of the Welsh Plant Breeding Station at Aberystwyth, Wales. Fifteen of these varieties, among them a variety of Black Mesdag, appeared to be quite susceptible. They also state that *U. levis* is very rare in Wales, having been found in the experimental plats only on *Avena nuda* L. var. *chinensis*, and *A. strigosa* Schreb. sub-species *glabrescens* and *orcadensis*. They inoculated eight varieties of oats with spores of *U. levis* from Orkney *strigosa* and obtained infection on this variety; the other seven varieties, however, Algerian *sterilis*, Welsh *strigosa*, Ceirch du Bach, Black Tartar, Golden Rain, Radnorshire Sprig, and Potato, gave negative results, although according to their other records these varieties are more or less severely attacked by *Ustilago avenae*.

In his further varietal tests for resistance of oats to loose and covered smut the writer has endeavored to get together varieties from as many regions as possible. A number of varieties were obtained from Director R. G. Stapledon of the Welsh Plant Breeding Station, Aberystwyth, Wales. At the same time, specimens of *Ustilago avenae* and *U. levis* also were secured from this station. These varieties of oats have been used in further tests, and a comparison also has been made between the Welsh smuts and those previously used by the writer.

In 1923, separate lots of seed of a number of varieties of oats were inoculated with the spores of *Ustilago avenae* and *U. levis* collected in my

experimental plots of previous years and also from the specimens obtained from Wales. These seeds were planted in adjacent plats in the experimental field. The results are given in table 1.

The varieties Black Mesdag (S. N. 70 and S. N. 594), Fulghum (S. N. 129), and Red Algerian (S. N. 597) gave negative results, or nearly so, with all the smuts. The oat varieties from Wales, Irish Tan (S. N. 582) and Sandy (S. N. 598), gave fairly high infections with both smuts from Missouri<sup>2</sup> and with *Ustilago avenae* from Wales; they all, however, gave negative results with *U. levis* from Wales. Two subspecies of *A. strigosa* (S. N. 587 and S. N. 592) were inoculated with *U. avenae* and *U. levis* from Wales. Practically negative results were obtained with the former smut, but very high percentages were secured with the latter. *Avena nuda* var. *inermis* (S. N. 30) gave very high percentages of infection with *U. avenae* and *U. levis* from Missouri but negative results with both smuts from Wales. The variety Victor (S. N. 126) showed a fairly high percentage of infected plants in all of the series.

In 1924 a series of experiments was started in the greenhouse under as carefully controlled conditions as possible. Separate lots of seed of 18 varieties of oats were inoculated with spores of loose and covered smut from Missouri and also from Wales. The inoculated seed was planted in sand with a soil moisture of 20 percent of its water-holding capacity and covered to the depth of one inch. The seed was germinated in the constant-temperature tank at 20° C. A sand moisture of 20 percent and a temperature of 20° C. have proved to be very favorable for securing high percentages of infection with both *Ustilago avenae* and *U. levis*. As soon as the seedlings had emerged from the sand they were transferred to the greenhouse benches. The data are given in table 1.

It is specially important to emphasize the fact that unusually high percentages of infection were obtained with every oat smut on one or more varieties of oats. In most cases the number of plants grown was sufficiently large, and the infection of all the plants inoculated was obtained in a great many cases. Evidently favorable conditions for severe infection were secured, and the evidence for host specialization on the part of these smuts is quite conclusive.

*Ustilago avenae* from Missouri. This loose smut gave 100 percent infection, or nearly so, on a large number of varieties, including *Avena nuda* L. var. *inermis* (S. N. 30), *A. sativa* L. var. Canadian (S. N. 119), Irish Tan (S. N. 582), Potato (S. N. 583), Radnorshire Sprig (S. N. 584), Victor (S. N. 126), *A. sativa orientalis* L. var. Black Tartar (S. N. 585), and the three sub-species of *A. strigosa* (S. N. 587, 589, and 592). Only 34.6 percent of the plants of Sandy (S. N. 598) and 12.5 percent of the plants of Monarch (S. N. 161) were infected. Negative results were obtained on

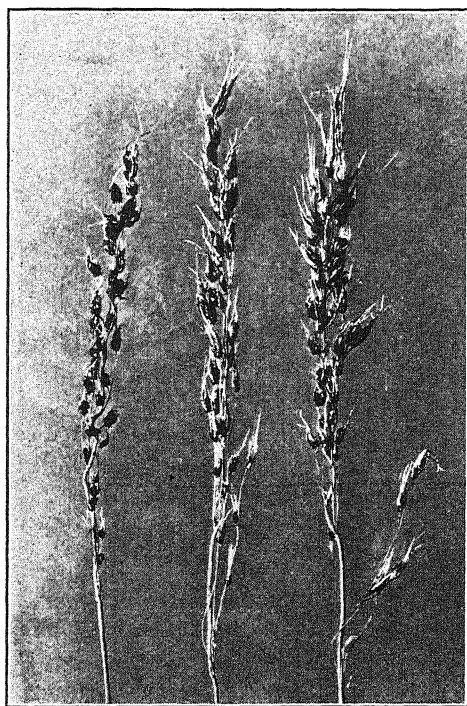
<sup>2</sup> The collections of *Ustilago avenae* and *U. levis* used by the writer in his experiments were originally collected in Missouri and hence are so designated.

TABLE I. *Comparison of Physiologic Races of Usilago Avenue (Pers.) Jens. and U. levis (K. & S.) Magn.*

Species and Variety	Seed No.	Year	<i>Ustilago avenae</i>				<i>Ustilago levis</i>							
			Missouri		Wales		Missouri		Wales					
			Total No. Plants	No. Inf.	% Inf.	Total No. Plants	No. Inf.	% Inf.	Total No. Plants	No. Inf.	% Inf.			
<i>Avena brevis</i> Roth.	289	1924	48	0	0	43	0	0	40	1	2.5	37	37	100.0
<i>Avena nuda</i> L. var. <i>inermis</i> .	30	1923	111	98	88.2	30	0	0	108	105	97.2	24	24	0
<i>Avena sativa</i> L.	30	1924	43	43	100.0	41	1	2.4	35	27	77.1	44	44	0
var. <i>Black Mesdag</i> .	70	1923	125	0	0	40	0	0	126	0	0	40	40	0
" <i>Black Mesdag</i> .	70	1924	43	0	0	43	0	0	45	0	0	38	0	0
"	594	1923	75	1	1.3	70	0	0	77	0	0	09	0	0
" <i>Canadian</i> .	594	1924	41	0	0	42	0	0	49	6	12.2	40	40	0
" <i>Irish Tan</i> .	119	1924	40	40	100.	46	43	93.4	36	36	100.0	48	48	0
"	582	1923	78	28	35.8	72	18	25.0	83	40	48.1	53	53	0
"	582	1924	42	42	100.0	39	39	100.0	7	5	71.4	47	47	0
" <i>Monarch</i> .	161	1924	32	4	12.5	42	0	0	35	34	97.1	44	44	0
" <i>Potato</i> .	583	1924	26	26	100.0	45	45	100.	7	7	100.0	42	42	0
" <i>Radnorshire Sprig</i> .	584	1924	34	31	91.1	46	34	73.9	29	1	3.4	46	46	0
" <i>Sandy</i> .	598	1923	84	23	27.3	94	39	41.4	83	16	19.2	87	87	0
"	598	1924	26	9	34.6	43	42	97.0	44	7	15.9	37	37	0
" <i>Victor</i> .	126	1923	220	92	41.7	45	15	33.3	222	139	62.6	51	14	27.4
<i>Avena sativa orientalis</i> L. var. <i>Black Tartar</i>	126	1924	27	27	100.0	36	27	75.0	23	23	100.0	42	42	0
<i>Avena sterilis</i> L. var. <i>Fulghum</i> .	585	1923	59	5	8.4	75	1	1.3	80	8	10.0	07	0	0
"	585	1924	28	28	100.0	44	28	63.6	18	15	83.3	46	46	0
" var. <i>Red Algerian</i> .	129	1923	212	0	0	48	0	0	217	0	0	39	39	0
"	597	1924	—	—	—	46	0	0	61	—	—	40	40	0
<i>Avena strigosa</i> Schreb.	597	1923	68	0	0	71	1	1.4	61	0	0	73	73	0
<i>Avena strigosa</i> Schreb.	597	1924	15	0	0	40	0	0	11	0	0	40	40	0
sub-sp. <i>glabrescens albida</i> .	133	1924	45	0	0	41	0	0	37	0	0	43	43	0
"	587	1923	—	—	—	24	1	4.1	—	—	—	18	15	83.3
" <i>orcadenensis flava</i> .	587	1924	44	43	97.7	45	0	0	24	11	45.8	45	45	100.0
" <i>pilosa alba</i> .	589	1924	43	43	100.0	39	0	0	21	21	100.0	35	35	100.0
"	592	1923	—	—	—	7	0	0	—	—	—	9	9	100.0
"	592	1924	40	40	100.0	38	0	0	30	26	86.6	46	46	100.0



Black Mesdag (S. N. 70 and 594), Red Algerian (S. N. 597), *A. brevis* (S. N. 289), and *A. strigosa* (S. N. 133). These results are quite in line with all those previously obtained with this smut on these varieties.



TEXT FIG. 1. *Ustilago avenae* (Pers.) Jen. on *Avena strigosa* Schreb. sub-sp. *pilosa*.

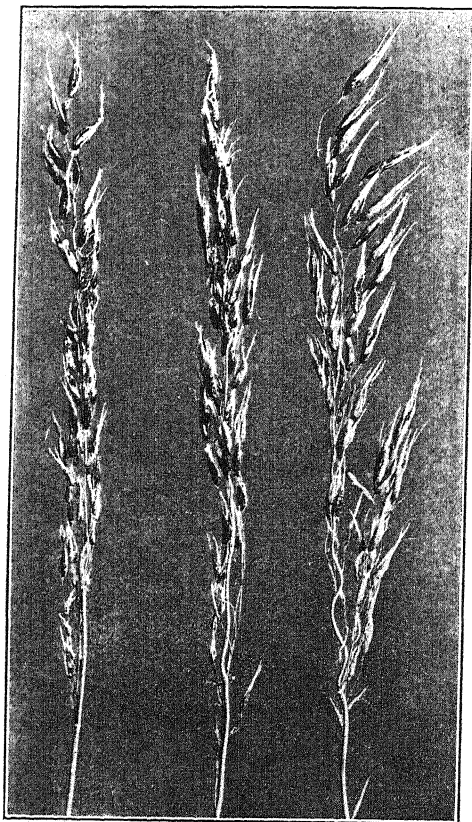
*Ustilago avenae* from Wales. The loose smut from Wales also gave very high percentages of infection on Canadian (S. N. 119), Irish Tan (S. N. 582), Potato (S. N. 583), Radnorshire Sprig (S. N. 584), Sandy (S. N. 598), and Victor (S. N. 126). Fairly high percentages of infection also were obtained on Black Tartar (S. N. 585). Negative results were secured with *Avena brevis* (S. N. 289), Black Mesdag (S. N. 70 and 594), Monarch (S. N. 161), Fulghum (S. N. 129), Red Algerian (S. N. 597), and all the sub-species of *A. strigosa*. Only one plant of *A. nuda* var. *inermis* (S. N. 30) out of a total of 41 was infected. It is possible that the infection of this plant was due to spores of the Missouri strain of *Ustilago avenae* which had accidentally come in contact with the seed.

The most significant differences between the loose smut from Missouri and that from Wales are found in the failure of the latter to cause a severe infection of *Avena nuda* (S. N. 30) and of the Welsh varieties of *A. strigosa* (S. N. 587, 589, and 592). The variety Monarch also gave negative results.



On the other hand, Sandy was severely attacked by the Welsh loose smut as compared with the Missouri race.

*Ustilago levis* from Missouri. The covered smut from Missouri gave very high percentages of infection on *Avena nuda* (S. N. 30), Canadian (S. N. 119), Irish Tan (S. N. 582), Monarch (S. N. 161), Potato (S. N. 583), Victor (S. N. 126), Black Tartar (S. N. 585), and the three sub-species of

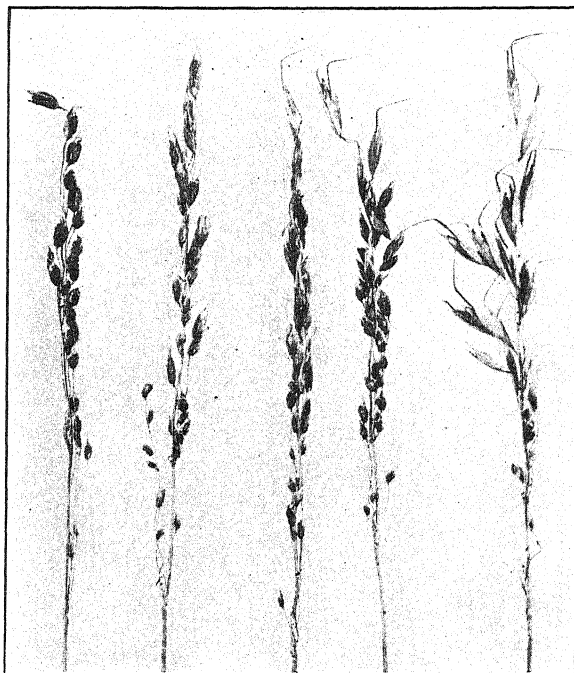


TEXT FIG. 2. *Ustilago levis* (K. & S.) Magn. on *Avena strigosa* Schreb. sub-sp. *pilosa*.

*A. strigosa* (S. N. 587, 589, and 592). Negative results were obtained on Black Mesdag (S. N. 70), Red Algerian (S. N. 597), and *A. strigosa* (S. N. 133). One plant of *A. brevis* (S. N. 289) out of a total of 40 was infected. Six plants of Black Mesdag (S. N. 594) out of a total of 49, one plant of Radnorshire Sprig (S. N. 584) out of a total of 29, and seven plants of Sandy (S. N. 598) out of a total of 44 also were infected. These results are quite in harmony with those previously recorded for this race. The data indicate that the covered smut from Missouri corresponds somewhat closely to the loose smut from Missouri in its capacity for infecting varieties of oats. The

most important differences are found in its ability somewhat to infect *A. brevis* and severely to attack Monarch (S. N. 161). Radnorshire Sprig has also shown high resistance to this smut. Black Mesdag (S. N. 594) appears to be somewhat susceptible.

*Ustilago levis* from Wales. The covered smut from Wales produced 100 percent infection on *Avena brevis* (S. N. 289) and on the three subspecies of *A. strigosa* (S. N. 587, 589, and 592) (see text fig. 2). All the other



TEXT FIG. 3. *Ustilago levis* (K. & S.) Magn. on *Avena brevis* Roth.

varieties of oats gave negative results in the greenhouse experiment. The race evidently differs very markedly from all the other three. In addition to its capacity for severely attacking the varieties of *A. strigosa* from Wales, its most surprising feature is its ability to produce 100 percent infection upon *A. brevis*. Further, the plants were entirely smutted, no sound heads being produced.

In previous years a few plants of *Avena brevis* (S. N. 289) have been observed to be infected by *Ustilago levis* from Missouri (text fig. 3). In 1921 two plants out of a total of 87 (2.3 percent), in 1922 two plants out of a total of 454 (0.4 percent), and in 1923 ten plants out of a total of 60 (16.6 percent) were observed to be infected. In every case the plants attacked were only partially smutted, tall normal stalks being produced

together with one or more dwarf smutted stalks. During the same three years, 706 plants of this oat inoculated with spores of *U. avenae* from Missouri also were grown but none of them were found to be infected.

The Missouri races of *Ustilago avenae* and *U. levis* have shown a difference in their behavior on Monarch (S. N. 161). Table 2 gives a comparison of the two smuts for the three seasons 1921, 1922, and 1923.

TABLE 2. *Susceptibility of Avena sativa L. var. Monarch (S. N. 161) to Loose and Covered Smut*

Years Grown	<i>Ustilago avenae</i>			<i>Ustilago levis</i>		
	Total No.	No. Inf.	Percent Inf.	Total No.	No. Inf.	Percent Inf.
1921.....	128	14	10.9	88	43	48.8
1922.....	134	1	.7	83	35	42.5
1923.....	76	1	1.3	76	54	71.0

It will be observed from this table that the Monarch variety is very much more susceptible to *Ustilago levis* than it is to *U. avenae*. The results obtained in the present experiments are quite in harmony with the previous data. It is, however, further interesting that this variety has given negative results with both strains from Wales.

It is evident from the data obtained that there are distinct specialized races of *Ustilago avenae* and *U. levis*. Certain varieties of oats appear to be resistant to all the races which have been studied, namely, Black Mesdag (S. N. 70), Fulghum (S. N. 129), Red Algerian (S. N. 597), and *Avena strigosa* (S. N. 133).

*Ustilago avenae* from Missouri is characterized by its capacity for severely infecting several American and Welsh varieties of *Avena sativa*. It also severely attacks *A. nuda* var. *inermis* (S. N. 30) and certain sub-species of *A. strigosa* which have been obtained from Wales.

*Ustilago avenae* from Wales also is characterized by its ability to infect a number of American and Welsh varieties of *Avena sativa*. It differs, however, from the preceding race in producing practically no infection on *A. nuda* (S. N. 30) and on the sub-species of *A. strigosa*.

*Ustilago levis* from Missouri is quite similar in its behavior to *U. avenae* from Missouri. It differs, however, in being able slightly to infect *Avena brevis* (S. N. 289) and to a very marked extent Monarch (S. N. 161). Some other differences in its behavior toward certain varieties may also be observed.

*Ustilago levis* from Wales is differentiated by its ability to infect severely *Avena brevis* (S. N. 289) and also the sub-species of *A. strigosa* (S. N. 587, 589, and 592). Negative results were obtained on all of the other varieties which were grown. In the field experiment of 1923, however, a considerable number of infected plants of Victor (S. N. 126) were observed. No

explanation is available as to why these positive results have not been confirmed in the more accurate experiment carried on in the greenhouse.

The important question arises as to the probable distribution of specialized races of the oat smuts. In all of his previous work on varietal resistance the writer has used the same strains of oat smuts which were originally collected in Missouri. No special effort previously had been made to determine the existence of specialization, except that in 1921 an experiment was carried out with four collections of *Ustilago levis*. These came from rather widely separated localities especially as compared with the original Missouri strain: Skagway, Alaska; McMinnville, Oregon; Mt. Vernon, Washington; and Bozeman, Mont. Separate lots of seed of five varieties of oats were inoculated: *Avena nuda* (S. N. 30), Black Mesdag (S. N. 117), Canadian (S. N. 119), Victor (S. N. 126), and Fulghum (S. N. 129). Black Mesdag and Fulghum gave negative results with all the collections. Comparatively high infections were obtained with all of them on *A. nuda*. Canadian and Victor also gave fairly high percentages of infection. The collection from McMinnville, Oregon, consistently gave the lowest results. The results do not clearly indicate any definite evidence of specialization. Variations in infection occurred, but these were not particularly different from those obtained in ordinary field plantings with oats. It is possible that, if a larger number of varieties of oats had been included in this test, distinct differences would have become apparent.

It may be that the explanation of the resistance of Kherson oats in the experiments of Heald (2) as compared with its susceptibility in the writer's experiments is that there is a real difference in the infecting capacity of the smuts used. It must, however, be emphasized that strains of oat varieties may differ in their susceptibility, and it is important to make careful comparisons with identical strains or with pure lines.

A particularly interesting question also comes up in connection with the cultivated varieties of *Avena sterilis* L. The varieties of this species, such as Fulghum, Burt, Red Rustproof, and Red Algerian, consistently have shown a high degree of resistance to the oat smuts in the writer's experiments. Field data indicate, however, that oat smut is somewhat of a problem in the southern United States where these varieties are grown extensively. It may well be that there are distinct specialized races of oat smuts which may be found in the southern United States and which are characterized, in part at least, by their capacity for infecting the *sterilis* varieties.

The fact that the first evidence for specialized races in the oat smuts was obtained by comparing smut collections from Wales and from Missouri does not necessarily indicate that such races are widely separated geographically. It is just as probable that they may be isolated from the same locality or even from the same field. In fact, Faris (1) obtained two distinct races of *Ustilago hordei* from the same collection of smut. In this particular

case smutted heads of two different varieties of barley had been collected together, and his experiments demonstrated that one variety harbored one race of smut and the other variety another race.

It is also interesting to note that somewhat similar results on varietal resistance have been obtained in widely separated regions. Vavilov (8) in Russia found Black Mesdag quite resistant to *Ustilago avenae*. His other results with a large number of varieties of oats are quite in line with those secured by the writer. Kulkarni (3) also has grown in India certain varieties of sorghum which the writer (6) has found to be either susceptible or resistant to *Sphacelotheca sorghi*. Using an Indian collection of this covered kernel smut, Kulkarni obtained results quite in harmony with those previously reported. There may be, however, real differences between these collections of covered smut of sorghum which would become apparent only on trial with a large number of varieties of the host. Careful comparisons with the same varieties of hosts and smuts are necessary to determine the occurrence and extent of specialization.

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## THE EFFECT OF FLUCTUATIONS IN THE $\text{CO}_2$ CONTENT OF THE ATMOSPHERE ON THE RATE OF RESPIRATION OF LEAVES

H. A. SPOEHR AND J. M. MCGEE

(Received for publication October 23, 1923)

There exists at the present time no adequate method of determining either the rate of respiration or that of photosynthesis of a plant under entirely normal conditions. It is necessary to confine a plant or a portion of a plant, such as a leaf, in a relatively small volume. The gaseous exchange of the plant can then be determined either by continuous displacement of the air surrounding the plant or by determination of the change of composition of the atmosphere when the plant is kept in a confined space. The principle applies not only to land plants in air, but also to aquatic plants surrounded by water.

Although most of the evidence permits the conclusion that under such experimental conditions the photosynthetic and respiratory activities are the same as under normal conditions in nature, recent experience has shown that great care must be exercised in interpreting the results of the rates of gas exchange obtained under such experimental conditions. The purpose of this paper is to demonstrate the effect of changes in the  $\text{CO}_2$  concentration of the air on the apparent rate of respiration of excised leaves.

In measuring the rate of respiration or photosynthesis under different conditions of carbon-dioxid content of the air about the leaf, the correct interpretation of results depends upon an exact knowledge of the manner in which the plant or leaf responds to a change in these external conditions. In determining the rate of photosynthesis on the basis of the  $\text{CO}_2$  fixed, it is necessary also to determine the rate of respiration. It has been very generally assumed that the latter, determined in the dark, remains the same during illumination. A correct estimation of the rate of photosynthesis depends, therefore, largely upon an exact determination of the respiratory value. Moreover, for practical experimental purposes it is often desirable that the photosynthesis determinations be made in an atmosphere enriched in  $\text{CO}_2$ . The rate of respiration is very often determined by measuring the  $\text{CO}_2$  emitted in a definite period of time when a stream of air free

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of  $\text{CO}_2$  is drawn over the plant. In general, photosynthesis tends to decrease the  $\text{CO}_2$  concentration of the surrounding medium, while respiration has the opposite effect. In a confined space it is impossible to keep the concentration of  $\text{CO}_2$  constant; in fact, it is by means of the changes in  $\text{CO}_2$  concentration that the respiratory and photosynthetic activities are measured, while in an open system in which the air or dissolved  $\text{CO}_2$  is continually displaced, a constant  $\text{CO}_2$  concentration can be maintained only by a very rapid stream, which, in turn, makes the complete absorption of the  $\text{CO}_2$  in the air stream impossible. Avoiding a variation in the concentration of the  $\text{CO}_2$  surrounding the leaf is thus experimentally high impossible. The question then arises whether such variations in the  $\text{CO}_2$  concentration of the surrounding medium are of consequential influence on the determination of the rates of respiration and photosynthesis.

The manner in which photosynthesis is influenced by different concentrations of  $\text{CO}_2$  has been quite definitely established. The effect of varying concentrations of  $\text{CO}_2$  on the rate of respiration is not so well known. That such variations have a decided influence on the rate of  $\text{CO}_2$  emission of a leaf is demonstrated by the following experiments. The results are of particular importance in the interpretation of experimental data.

#### APPARATUS AND METHODS

The experimental material consisted of single excised leaves of *Helianthus annuus*, of *Echinocystis fabacea*, and of *Hydrangea hortensis*, which were cut in the manner already described.<sup>1</sup> The apparatus employed was of the same type as that used in previous work, with certain important changes. The method of  $\text{CO}_2$  determination was that described in a previous article.<sup>2</sup> A new leaf chamber (text fig. 1) was used, consisting of a brass frame, 15 by 25 cm., two sides of which were glass plates 5 mm. apart. The cover was made of solid brass and was held in place by two wing-nuts screwed to threaded brass posts passing through holes in the cover. An air-tight seal was made by means of a heavy rubber sheet, which was tightly pressed between the frame and cover when the wing-nuts were screwed down. The glass plates were firmly held in place in the frame by means of a cement of litharge and glycerin. This cement was coated over with a water-proof layer of varnish (valspar) on the outside where it came in contact with the water of the thermostat.

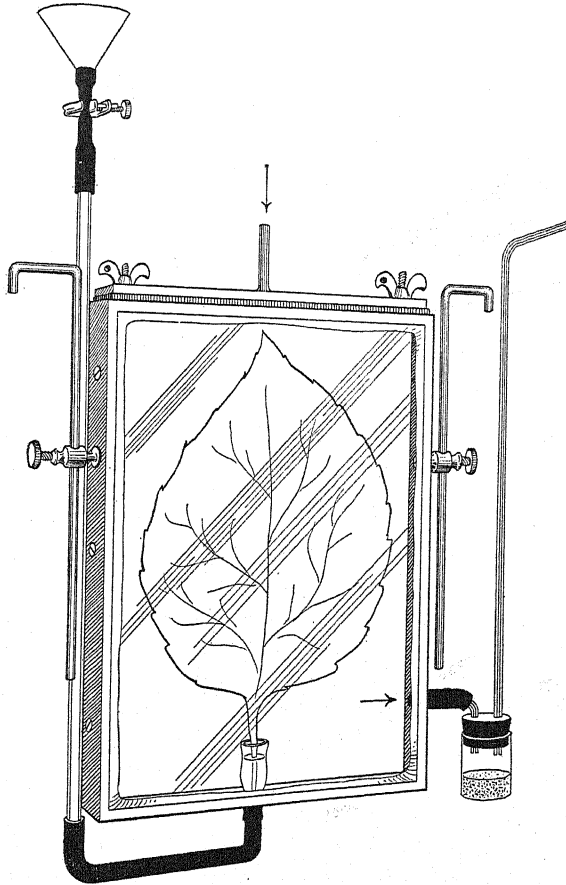
In place of the aspirator used in former experiments, a new one, consisting of two large bottles of 30-liters capacity, was substituted; each bottle having an opening near the base as well as in the neck. One was placed upon the table and was fitted up as a "Mariotte-bottle" aspirator, while the other was placed upon the floor and was the receiving bottle. It was fitted with a gauge attached at the side-neck, so that the amount of water

<sup>1</sup> Spoehr, H. A., and McGee, J. M. Carnegie Inst. Wash. Publ. 325: 31, 84. 1923.

<sup>2</sup> Same, Industrial and Engineering Chem. 16: 128. 1924.



it contained at any time could be measured. In this way, the amount of air which had entered the upper bottle during any period of time could be estimated by reading the volume of water in the lower bottle at the beginning and end of the period. The outflow tube of the "Mariotte bottle" led from the side-neck at the base through a rubber stopper in the upper neck of the receiving bottle. When the water in the "Mariotte bottle"



TEXT FIG. 1. Container for excised leaf used in experiments on respiration and photosynthesis. The petiole of the leaf is in a nutrient solution; the air stream enters at the top, leaves at the lower edge of the frame, and is dried over  $P_2O_5$  before passing through the absorption tubes.

had run out, it was refilled by forcing the water back into it by means of air pressure applied to the receiving bottle.

It is very essential to a method for determining the rate of respiration or photosynthetic activity, based upon the differential determination of  $CO_2$  by absorption in  $Ba(OH)_2$  solution, that the air stream passing over

the leaf be as accurately under control as possible. This is especially true when air is used to which  $\text{CO}_2$  has been added. A slight variation in the rate of the air stream may introduce an error many times greater than any change in the rate of emission or absorption of  $\text{CO}_2$  by the leaf. The best control that could be found was a fine needle valve that pierces just through a piece of lead. Hence, throughout the investigation the greatest care was taken to maintain a uniform air stream (1000 cc. per hour). The thermostat remained at  $25^\circ \pm 0.05^\circ$ .

After each experiment the leaf was removed from the leaf-container and placed in a photographic printing frame with blue-print paper. From the print thus produced the area of the leaf was determined by means of a planimeter, and the rates of emission of  $\text{CO}_2$  per 100 sq. cm. were calculated.

RATE OF RESPIRATION IN  $\text{CO}_2$ -FREE AIR AND IN AIR CONTAINING 0.6 PERCENT  $\text{CO}_2$

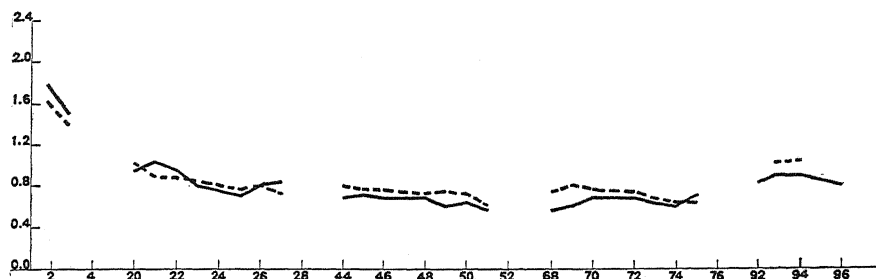
In most of the work which has been done on the effect of  $\text{CO}_2$  on the respiration of plants, relatively high concentrations of this gas were employed. It was of interest, therefore, first to determine whether the concentrations of  $\text{CO}_2$  used in the photosynthesis experiments exerted any influence on the respiratory activity. For this purpose two leaves of *Helianthus annuus* adjacent on the same plant were selected. They were allowed the same amount of illumination before being cut, were cut at the same time of day, and in every way were so treated that their condition at the beginning of the time of observation might be as nearly alike as possible. These leaves were cut from the plant and placed in the leaf frame in the manner described in a former publication.<sup>3</sup>

A comparison was made of the rate of respiration of these leaves, one in a  $\text{CO}_2$ -free air stream, the other in an air stream containing twenty times the  $\text{CO}_2$  ordinarily in the atmosphere, i.e., about 0.6 percent. The rate of respiration was determined for a number of one-hour periods per day, and these determinations were continued for four days. In this manner, the rate-of-respiration curve of the first leaf was determined in an air stream from which the  $\text{CO}_2$  had been removed by drawing it through moist soda lime. Then that of the second leaf was determined in an air stream of which the  $\text{CO}_2$  content had been increased. The rate in each case was found to decrease rapidly during the first 24 hours and then to remain fairly constant until the leaf had been under observation about 90 hours. The results of these observations are shown graphically in text figure 2. For reasons of economy of space it has been necessary to omit the numerical data of the experiments in this publication. However, the authors will be glad to send the protocol of these experiments to any one interested in them.

Allowing for the individual differences in the two leaves, it will be seen from the graph that the rate of respiration of a leaf is not appreciably differ-

<sup>3</sup> Spoehr, H. A., and McGee, J. M. Carnegie Inst. Wash. Publ. 325: 31, 85. 1923.

ent in character in air containing 0.6 percent  $\text{CO}_2$  by volume from that in air free of  $\text{CO}_2$ .



TEXT FIG. 2. Rate of respiration of an excised leaf of *Helianthus annuus*. The solid line gives the rate in air free of  $\text{CO}_2$ , the broken line in air containing 0.6 percent  $\text{CO}_2$ . The ordinate represents mg.  $\text{CO}_2$  emitted per 100 sq. cm. leaf surface; the numbers on the abscissa represent time in hours when  $\text{CO}_2$ -emission determinations were made.

#### THE EFFECT OF VARIATIONS IN THE $\text{CO}_2$ CONTENT OF THE AIR STREAM ON THE RATE OF $\text{CO}_2$ EMISSION

Since, in investigations on photosynthesis, the  $\text{CO}_2$  content of the air surrounding a leaf or plant changes as  $\text{CO}_2$  is absorbed by the leaf, it was necessary to determine what effect this change would have upon the subsequent rate of emission of  $\text{CO}_2$  by the leaf. For this purpose, leaves of *Helianthus annuus* were used. A leaf, the initial rate of respiration of which had been determined in  $\text{CO}_2$ -free air, was kept in an air stream free of  $\text{CO}_2$  for 43 hours. At the end of that time, air enriched with  $\text{CO}_2$  was drawn through the system. This was continued for a period of one hour before determinations of the rate of respiration were made, in order to make certain that all of the  $\text{CO}_2$ -free air in the apparatus had been displaced by air containing a known quantity of  $\text{CO}_2$ . It had been ascertained by a preliminary experiment that the apparatus could be swept clear of  $\text{CO}_2$  in twenty minutes. Hence an hour was considered ample time to insure the complete replacement of one air stream by the other.

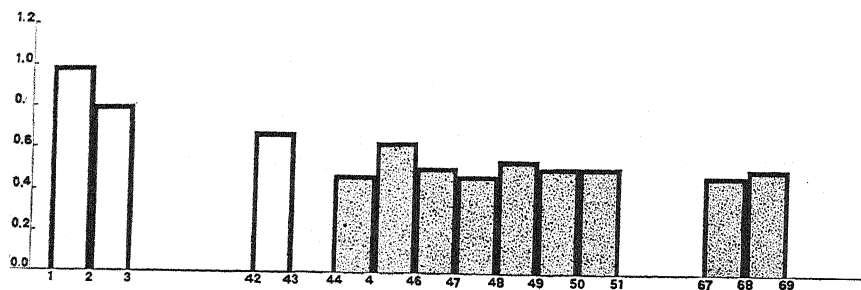
The determinations of the rate of respiration of the leaf in the air stream containing  $\text{CO}_2$  were made for seven hours consecutively, and again, after an interval of 16 hours, for two one-hour periods. These determinations showed the effect upon the rate of emission of  $\text{CO}_2$  of an increase in the  $\text{CO}_2$  content of the air about a leaf. The result is shown graphically in text figure 3.

As will be seen from the graph, the rate of emission of  $\text{CO}_2$  dropped during the first hour after the air stream had been changed, then increased and continued at approximately the same rate as before the  $\text{CO}_2$  content of the air had been increased.

This experiment was repeated, using another leaf of *Helianthus annuus*. In the second experiment, the leaf was kept in the dark in  $\text{CO}_2$ -free air for

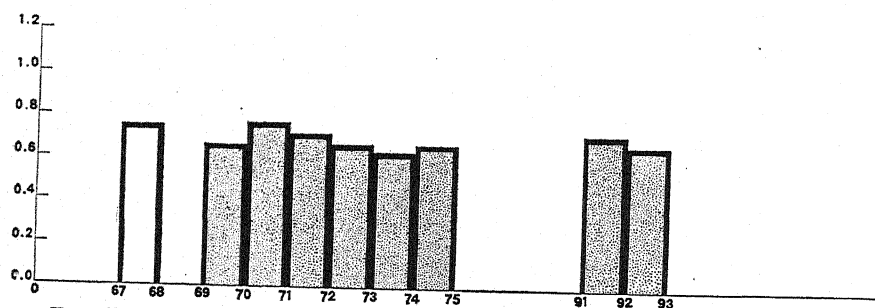
68 hours before the apparatus was filled with air to which  $\text{CO}_2$  had been added. The results of this experiment are given in text figure 4. These results show the same effect from the change in concentration of the  $\text{CO}_2$ .

In order to determine if this effect was to be observed with leaves from other plants, similar experiments were carried out with leaves of *Echinocystis fabacea*. A determination of the moisture in these leaves showed them



TEXT FIG. 3. The reduction in the rate of  $\text{CO}_2$  emission of a leaf of *Helianthus annuus* when the air stream is changed from  $\text{CO}_2$ -free to 0.6 percent  $\text{CO}_2$ . The ordinate represents mg.  $\text{CO}_2$  emitted per 100 sq. cm. leaf surface; the numbers on the abscissa represent time in hours when  $\text{CO}_2$ -emission determinations were made. The shaded areas represent rate of  $\text{CO}_2$  emission in 0.6 per cent  $\text{CO}_2$ , the unshaded in  $\text{CO}_2$ -free air.

to contain approximately the same amount of water as the sunflower leaves, although the habits of the two plants are quite different. The effect upon the rate of emission of  $\text{CO}_2$  of a sudden change in the  $\text{CO}_2$  content of the



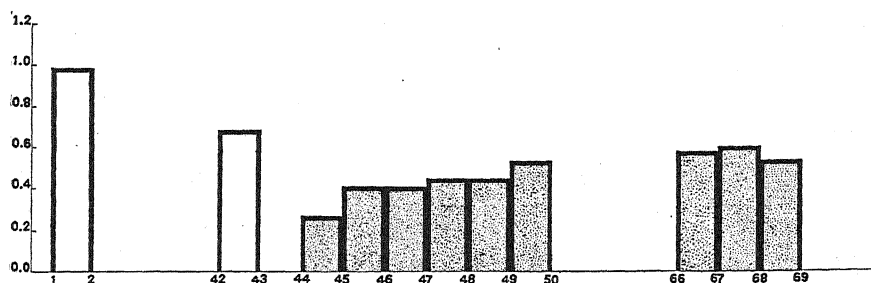
TEXT FIG. 4. The reduction in the rate of  $\text{CO}_2$  emission of a leaf of *Helianthus annuus* when the air stream is changed from  $\text{CO}_2$ -free to 0.6 percent  $\text{CO}_2$ . The ordinate represents mg.  $\text{CO}_2$  emitted per 100 sq. cm. leaf surface; the numbers on the abscissa represent time in hours when  $\text{CO}_2$ -emission determinations were made. The shaded areas represent rate of  $\text{CO}_2$  emission in 0.6 percent  $\text{CO}_2$ , the unshaded in  $\text{CO}_2$ -free air.

air surrounding a leaf of this plant was more pronounced, but of the same nature as that of the sunflower leaf, and the evolution of  $\text{CO}_2$  did not return to a constant rate for several hours. The data from these experiments are shown graphically in text figure 5.

A similar experiment was carried out with a leaf of *Hydrangea hortensis*. The rate of  $\text{CO}_2$  emission of this rather thick leaf during the first three

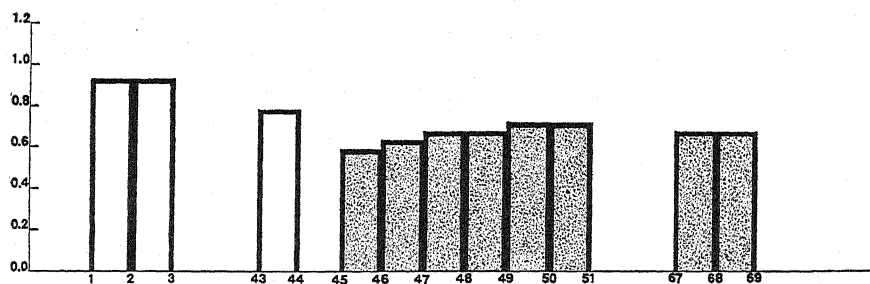
hours was considerably decreased after the change from an atmosphere free of  $\text{CO}_2$  to 0.6 percent  $\text{CO}_2$ . The results are shown graphically in text figure 6.

The behavior of a leaf when the reverse procedure was followed was next observed. After the initial rate of respiration in  $\text{CO}_2$ -free air had been determined, the leaf of *Helianthus annuus* was kept in the dark in an air



TEXT FIG. 5. The reduction in the rate of  $\text{CO}_2$  emission of a leaf of *Echinocystis fabacea* when the air stream is changed from  $\text{CO}_2$ -free to 0.6 percent  $\text{CO}_2$ . The ordinate represents mg.  $\text{CO}_2$  emitted per 100 sq. cm. leaf surface; the numbers on the abscissa represent time in hours when  $\text{CO}_2$ -emission determinations were made. The shaded areas represent rate of  $\text{CO}_2$  emission in 0.6 percent  $\text{CO}_2$ , the unshaded in  $\text{CO}_2$ -free air.

stream containing approximately 0.6 percent  $\text{CO}_2$  by volume for 18 hours, and its rate of respiration was determined for two one-hour periods. The air stream containing  $\text{CO}_2$  was then changed to air free of  $\text{CO}_2$ , and after one hour the rate of respiration in  $\text{CO}_2$ -free air was determined for six

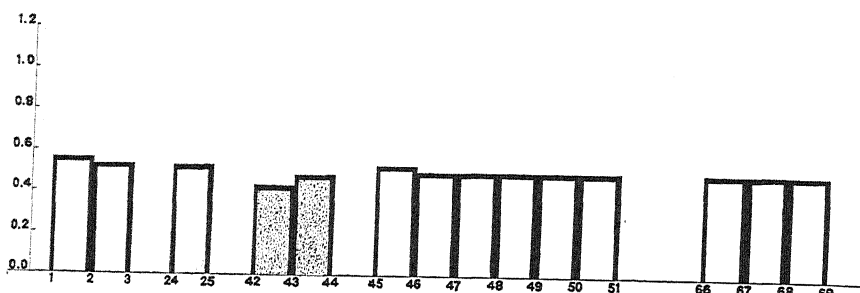


TEXT FIG. 6. The reduction in the rate of  $\text{CO}_2$  emission of a leaf of *Hydrangea hortensis* when the air stream is changed from  $\text{CO}_2$ -free to 0.6 percent  $\text{CO}_2$ . The ordinate represents mg.  $\text{CO}_2$  emitted per 100 sq. cm. leaf surface; the numbers on the abscissa represent time in hours when  $\text{CO}_2$ -emission determinations were made. The shaded areas represent rate of  $\text{CO}_2$  emission in 0.6 percent  $\text{CO}_2$ , the unshaded in  $\text{CO}_2$ -free air.

consecutive hours. Again, after an interval of 15 hours, the rate was determined for three one-hour periods. The data from this experiment are recorded in text figure 7.

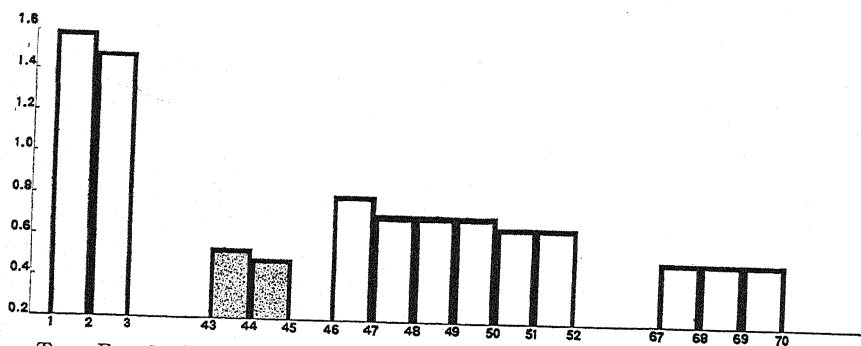
As is evident from these results, the change from an atmosphere of higher  $\text{CO}_2$  content to one of lower  $\text{CO}_2$  content results in an augmented rate of

CO<sub>2</sub> emission from the leaf, which emission after a period of an hour again attains a lower level.



TEXT FIG. 7. Variation in rate of CO<sub>2</sub> emission of leaf of *Helianthus annuus* with change in the CO<sub>2</sub> content of the air stream. The ordinate represents mg. CO<sub>2</sub> emitted per 100 sq. cm. leaf surface; the numbers on the abscissa represent time in hours when CO<sub>2</sub>-emission determinations were made. The shaded areas represent rate of CO<sub>2</sub> emission in 0.6 percent CO<sub>2</sub>, the unshaded in CO<sub>2</sub>-free air.

In the case of *Echinocystis fabacea* the difference in the rate of CO<sub>2</sub> emission under these conditions is considerably more marked and of longer duration, as can be seen from the results given in text figure 8.



TEXT FIG. 8. Variation in rate of CO<sub>2</sub> emission of leaf of *Echinocystis fabacea* with change in the CO<sub>2</sub> content of the air stream. The ordinate represents mg. CO<sub>2</sub> emitted per 100 sq. cm. leaf surface; the numbers on the abscissa represent time in hours when CO<sub>2</sub>-emission determinations were made. The shaded areas represent rate of CO<sub>2</sub> emission in 0.6 percent CO<sub>2</sub>, the unshaded in CO<sub>2</sub>-free air.

#### SUMMARY

When the CO<sub>2</sub> content of the air surrounding a leaf is changed from a lower to a higher concentration, the leaf shows a reduced rate of CO<sub>2</sub> emission for a period following the change, then increases, and finally again attains about the same rate as before the change in CO<sub>2</sub> content was made. Conversely, when the CO<sub>2</sub> content of the air surrounding a leaf is changed from a higher to a lower concentration, the leaf shows a primary increased rate of CO<sub>2</sub> emission and subsequent decrease to the original rate. The



intensity of this increased or decreased rate varies with different species of leaves, as does also the duration of the effect of the change.

#### DISCUSSION

These experiments show very decidedly that great care must be exercised in measuring the rate of photosynthesis by the rate of emission or absorption of  $\text{CO}_2$  by a leaf or plant, because a change in the  $\text{CO}_2$  content of the air surrounding the leaf is attended by phenomena that tend to change to a considerable extent the rate of emission from, or penetration of  $\text{CO}_2$  into, the leaf tissues. A high rate of photosynthesis reduces the  $\text{CO}_2$  in the atmosphere surrounding the leaf and results in a tendency for  $\text{CO}_2$  to diffuse out of the leaf into the atmosphere, thus showing an apparently lower rate of photosynthesis than is actually the case. This is especially true in a closed system, and when aquatic plants are being investigated where a slightly alkaline solution is used. The effect can be avoided either by using a very rapid air stream (which introduces the difficulty of complete  $\text{CO}_2$  absorption) or, in the case of aquatic plants, by employing a proper buffer solution. It also emphasizes the desirability of using thin leaves which adjust easily to changes in the  $\text{CO}_2$  content of the surrounding air.

These results can, in part at least, be interpreted on the basis of equilibrium phenomena, *i.e.*, that the rate of  $\text{CO}_2$  emission from the leaf is directly affected by the partial pressure of the  $\text{CO}_2$  of the air surrounding the leaf.

Another factor of great importance, however, is the absorption of  $\text{CO}_2$  by the leaf material itself, as has been discussed by Willstätter and Stoll.<sup>4</sup>

In view of the great importance of these phenomena to the interpretation of the mechanism of the photosynthetic process, special investigations on the absorption of  $\text{CO}_2$  by the leaf material have been carried out and will be reported on in another publication.

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CARMEL, CALIFORNIA

<sup>4</sup> Untersuchungen über die Assimilation der Kohlensäure, pp. 172-225. Berlin, 1918.



## THE PNEUMATHODES ON THE ROOTS OF THE OIL PALM (*ELAEIS GUINEENSIS* JACQ.)

CECIL YAMPOLSKY

(Received for publication November 26, 1923)

The term "pneumathode" was coined by Jost (4) to designate certain definite structures, outgrowths from the roots of the palm *Livistona australis*. These structures function, according to Jost, like stomata and lenticels and are organs of aëration (*Durchlüftungsorgane*). This observation of Jost's on *Livistona* was extended to other palms, and a large number of plant forms are now known to show pneumathodes. Jost also found pneumathodes on *Pandanus*, *Saccharum*, *Cyperus*, and *Luffa*.

Jost observed that a plant of *Livistona australis* which was growing in a large wooden tub produced a large number of upright growing structures 2 to 4 mm. in diameter and 5 mm. above the surface of the soil. A mealy appearing tissue, either in the form of rings or covering the whole surface of the protuding organ, characterized these bodies. The structures are side branches which arise endogenously as do secondary roots from the larger roots. They are as a rule negatively geotropic. Pneumathodes also appear below the ground. Jost was able to induce the formation of pneumathodes by excessive watering.

Breathing organs in plants had been described before Jost. Goebel (3) found breathing organs borne by the marsh plants *Sonneratia* and *Avicennia* in the form of side roots which were negatively geotropic. Schenck (7) described the presence of dimorphic roots in the mangrove plants *Avicennia tomentosa* and *Laguncularia racemosa*. Copeland (2) recently found pneumathodes on the adventitious roots of *Cocos nucifera*. In another publication (10) the presence of pneumathodes on the roots of the oil palm has been described, and in the following pages a more detailed account of the structures as they occur normally and as they appear under changed environmental conditions will be given.

For a complete understanding of the aëration system of the oil palm, the root system as a whole must be briefly discussed, and for this reason much that has been described earlier is now repeated. The oil palm possesses two kinds of roots, underground roots and adventitious roots. The underground roots are attached to the base of the trunk. They radiate in all directions and grow for considerable distances. Bücher and Fickendey (1) describe roots 15 M. long, and they also traced roots to a depth of 9 M. An old tree of *Elaeis* may have close to 10,000 roots attached to its base. The roots as they emerge are soft but very soon become tough and elastic,

and they persist long after they have ceased to function as living organs. The large numbers of roots with their tensile strength and branchings form a complete network, affording the tree an ideal anchorage. A healthy tree can withstand the severest storms, and uprooting of a tree is unknown.

The adventitious roots arise above the ground usually on older trees and in regions where there is much moisture. They may appear one meter above the ground. Ordinarily, however, they emerge a short distance above the ground. Branching in adventitious roots is not pronounced. Ultimately most of the adventitious roots grow down to and penetrate the soil.

In both types of roots conspicuously large root caps are present. The roots when they first emerge are very soft. Differentiation sets in very soon, a resistant hypodermis is formed, and finally the roots become woody. In the older roots the hypodermis reaches to within a few centimeters of the root tip. The hypodermis is impervious to water, so that the region of absorption is limited to the few centimeters at the root tip. The adventitious roots are brownish in color and very often have a shaggy appearance. This appearance is due to the rings of tissue which mark the successive places of attachment of the root cap. The distance between successive rings is variable and indicates periods of accelerated growth alternating with periods of protracted growth (Pl. XXXVII, fig. 9).

A conspicuous feature of a root when it is cut in cross section a little behind the growing point is the presence of radiating lacunae or air chambers (figs. 15, 17, Pl. XXXVIII). The origin of these chambers can be traced back to the cells nearer the root tip where differentiation is not so pronounced (10). These lacunae, seen in cross section as radiating chambers, run lengthwise in the root. Individual lacunae do not necessarily run the full length of the root, but they arise and end at various levels. Such chambers, extending (considered together) through the entire length of the root, result in a most efficient aërating system. Lacunae are present in all normal roots, underground and adventitious.

The hypodermis of the roots, we have noted, is quite impervious, and the few centimeters at the end of the root represent the active region of absorption. However, the oil palm in common with other palms is provided with pneumathodes which are found scattered all along the adventitious roots and more sparingly along the underground roots. These organs allow for a communication between the interior of the root and the surrounding medium.

#### PNEUMATHODES

Pneumathodes occur normally on the adventitious roots of the oil palm. Figure 10, Plate XXXVII, shows a portion of an adventitious root with several pneumathodes distributed over its surface. The pneumathodes are relatively small bodies which protrude a few millimeters above the surface of the root. They can, however, be observed with the

naked eye. In roots of greater circumference the pneumathodes are larger. In some instances they may become conspicuous organs (10).

The form that a pneumathode may take depends upon its age and also upon the presence or absence of a root cap after its emergence. A pneumathode is a modified secondary root, and when it is first formed it can not be distinguished from a young secondary root. Soon after its emergence, modifications set in which limit its elongation. The forms that a pneumathode may take are seen in figures 10-14, Plate XXXVII. Figure 11 represents a pneumathode in which the root cap has persisted, and the subsequent lignification that set in included also the cells of the root cap. As a result of such a condition, the epidermis, which in almost all the pneumathodes is split and reflexed, exposing the inner tissue, remains intact in this pneumathode. In this instance whatever passage of gases occurs is beneath the root cap in and out of the pneumathode.

Figures 12-14 represent the typical pneumathodes scattered over the adventitious roots. Here it is apparent that the root cap has been lost, the epidermis in part lost and reflexed, and the inner tissue exposed to the atmosphere. Such pneumathodes show a mealy tissue—the aërenchyma tissue which we shall discuss shortly. The solid black protruding structures in the figures show the central vascular bundle becoming more prominent as the surrounding tissue is sloughed off. The pneumathode of figure 14 is represented in its earlier condition by figure 12. In spite of the loss in aërenchyma tissue, a longitudinal section through such a pneumathode (fig. 14) shows the cells to be still living and apparently functioning.

#### STRUCTURE OF A PNEUMATHODE

A longitudinal section through a young pneumathode before it has forced its way through the epidermis of the mother root shows the typical root cap at the end, the plerome, the periblem, and the epidermis of the root. The tissue of the periblem shows the most pronounced subsequent modification. Surrounding the bundle is a layer of parenchymatous cells; this is followed by a zone of aërenchyma—thin-walled cells with intercellular spaces. Then follows another zone of parenchyma, and finally comes the epidermis (fig. 18, Pl. XXXVIII). As the pneumathode develops, the cells of the various zones undergo modification. The cells of the aërenchyma zone increase in size, their walls become thicker and take on a pronounced woody character, sclerenchymatous tissue developing in the parenchymatous zones on either side of the aërenchyma. A hypodermis begins to form from the base of the pneumathode and its formation proceeds towards the tip. Before the development of the hypodermis has advanced too far, the pressure from the rapidly enlarging aërenchyma bursts the epidermis where it is least resistant (at the tip). This epidermis is reflexed, giving a vase-shaped appearance to the structure. In such a condition the exposed part of the pneumathode has a white, mealy appearance.

It is in a cross section of a pneumathode that the difference between it and a secondary root that has remained unmodified becomes apparent. It may be repeated here that a conspicuous characteristic of a root in cross section is the presence of radiating air chambers or lacunae and that lacunae are present in roots of all ranks. In the pneumathodes there are no radiating air chambers. In contrasting figure 19, Plate XXXVIII, which represents a cross section of an old pneumathode, with figures 15 and 17, cross sections of secondary roots, the difference is quite obvious.

In the young pneumathode the cells of the aërenchyma which have undergone modification are more or less stellate in character. This is quite apparent where cells come in contact with adjacent cells and protuberances are sent out. The intercellular spaces are large and aëration is thus made more efficient, especially since this aërenchyma comes in direct contact with the atmosphere. The cell walls are not smooth; they show wart-like structures (fig. 22).

Figure 20, representing a cross section of a pneumathode on an underground root of the oil palm, shows the characteristic aërenchyma with thick-walled cells and large intercellular spaces present in the pneumathodes of the adventitious roots. In pneumathodes whose epidermis has been ruptured, the cells of the aërenchyma, as a result of released pressure, enlarge and push the surrounding tissues aside. A microscopical examination of such exposed tissue as that of the pneumathode shown in figure 13, Plate XXXVII, shows the loose arrangement of aërenchyma together with the surrounding parenchyma which may have been modified into sclerenchyma. Figure 21, Plate XXXVIII, representing a longitudinal section of an induced pneumathode on the underground roots, shows very strikingly the loose arrangement of the aërenchyma after the epidermis has been ruptured.

In the pneumathodes whose epidermis is not ruptured, a hypodermis is formed underneath the epidermis and the aërenchyma ultimately degenerates (fig. 19). The parenchyma on the outside and the inside of the aërenchyma zone develop respectively into stone cell and sclerenchymatous tissue. This is a condition that has been described (10) in the adventitious roots when modifications of cells occur.

Pneumathodes, then, occur normally on the adventitious roots of the oil palm and are organs of gas-exchange. The organs are not limited to the oil palm and the coconut palm. Many of the palms growing in the Botanical Gardens at Buitenzorg, Java, bear similar structures on their adventitious roots.

#### INDUCED PNEUMATHODES

During the course of my observations on the oil palm, I had occasion to keep young plants in glass jars containing water for a period of several weeks. I observed that the new roots which were put out during that time, instead of showing the typical secondary roots, showed a large number of

short, fluffy, white bodies growing at right angles to the mother root. They did not grow upright like the bodies described by Jost, and they showed no tendency to grow out of the water and thus come in contact with the free atmosphere. These bodies were readily identified as pneumathodes.

To test the influence of water on the production of pneumathodes, young plants were placed under different moisture conditions. Twelve plants were placed in glass jars and the roots were kept continually under water, twelve plants were potted in sand and were kept moderately moist, twelve were potted in sand and kept excessively moist, twelve were potted in rich soil and kept moderately moist, twelve were potted in rich soil and kept excessively moist. The experiments were conducted for a period of a month.

The plants kept under water and those kept excessively wet in rich soil and in sand produced the largest numbers of pneumathodes. The other two sets of plants produced but few pneumathodes. The largest number of pneumathodes was produced on those roots which were formed after the experiment was started. Figure 1, Plate XXXVII, shows the root system of one of the plants whose roots were kept in water for a month. When the plant was first placed in water the root which later showed the many pneumathodes was just emerging from the base of the stem. Secondary roots which were formed during the course of the experiment also produced many pneumathodes (figs. 1, 2). The relative distribution of the secondary roots and of the pneumathodes can be seen in figure 1. One of the roots in this figure shows a pneumathode similar to that shown in figure 11, which is from an adventitious root. In its structure it is like a pneumathode on an adventitious root.

An induced pneumathode when examined with the naked eye is seen to be made up of two distinct elements—a smooth, tubular part attached to the mother root and a white, mealy part at the free end. The tubular portion represents that part of the structure which still has its epidermis intact, and the mealy portion represents the part where the epidermis has been ruptured and reflexed, exposing the interior tissue. The length of the tubular portion is variable, as can be seen from figures 1, 3, and 4. The same figures show that the length of the mealy white portion is also variable. Figures 3 and 4 represent portions of roots at some distance from the tip, and near the tip, respectively. Here the pneumathodes show the tubular structure with collars at their ends. The collars represent the epidermis which has been reflexed after part of it has been ruptured. The variations in length of the tubular parts of the pneumathodes indicate that rupturing of the epidermis may occur at any time in the growth of the organ.

Elongation and growth of the exposed portion of the pneumathode goes on so that curious structures like the one shown in figure 8 result. The growing point is quite active. The irregular rings no doubt indicate the previous places of attachment of the root cap, which still persists as a



very conspicuous organ. The growing point is hidden under the root cap. Figures 6, 7, and 8 show pneumathodes with the mealy parts in various stages of growth. Figure 5 shows the epidermis just after it has been ruptured; the collar is not yet formed. The inner tissue has not yet expanded to give the irregular outline so plainly seen in figures 6, 7, and 8. The exposed tissue can readily be snapped or sloughed off, and some of the tubular structures appearing in figure 4 show that the mealy portion has been broken off at that level. The pneumathode in figure 3 with part of the vascular bundle protruding indicates that the tissue has been sloughed off.

#### STRUCTURE OF THE INDUCED PNEUMATHODES

Figure 15, Plate XXXVIII, is a cross section of a root on which many pneumathodes were formed. This section passes through two mature and two newly formed ones. It is also intended to indicate the relation of the pneumathodes to the air chambers in the mother root. The heavy black lines within the periblem on both sides of the mature pneumathodes represent the cells crushed by the pressure of the pneumathode as it forced its way through the tissues of the mother root on its way towards the outside. Figure 16 shows a cross section of a pneumathode so cut as to include a portion of a longitudinal section of a mother root. It is quite evident that gas movement is facilitated by so loose an arrangement of cells. The heavy black lines show the zones of crushed cells.

A section of a developing pneumathode before the epidermis has been ruptured (fig. 20) shows the changes that have occurred in the aërenchyma. The cells have enlarged and have become thick-walled, and the intercellular spaces are conspicuous. Conspicuous too are the large cells containing bundles of raphides of calcium oxalate. Many of the cells of the aërenchyma also contain such crystals. The formation of a thick zone of aërenchyma is not sudden. Sections through many pneumathodes show that isolated groups of cells in the thin-walled aërenchyma have thick walls. This condition spreads until the aërenchyma is entirely modified.

It is interesting to contrast the conditions found in a cross section of a secondary root (fig. 17) formed on the same root as the pneumathode of figure 20. The distinct root character with its lacunae is seen in figure 17. The sclerenchyma tissue is quite pronounced, and a hypodermis is being produced. A cross section through the mealy-appearing part of a pneumathode would give a picture similar to that shown in figure 20 but with the epidermis removed. Such a section shows an irregular circumference composed of thick-walled aërenchyma. When the pressure of the growing and developing aërenchyma cells is sufficiently great, the epidermis is ruptured and in consequence the interior tissue is released from the counter-pressure and the ragged appearance of the end of the pneumathode is produced. Figure 21 is a longitudinal section through a pneumathode. It brings out strikingly the condition of the various tissues of the organ. The embryonic regions

of both the root cap and the root tip are active. The aërenchyma is irregularly disposed and branches freely. The cells are more or less stellate, so that large intercellular spaces result. The free ends of the cell rows give the mealy appearance to the whole structure. The giant character of the cells containing calcium-oxalate crystals is adequately brought out here. At the bottom of the figure the curved portions represent parts of the reflexed epidermis. Figure 22 shows the wart-like outgrowths from the cell walls of the aërenchymous tissue.

In their anatomical structure, these induced pneumathodes agree with the descriptions and figures of Jost (4). They do not, however, grow upright, and therefore they are not negatively geotropic. It is interesting to note that these pneumathodes are produced in greatest numbers when the root system is completely submerged.

#### DISCUSSION

Jost says that the function of the pneumathodes is that of aëration, like that of stomata and lenticels. Schenck (7) says that aërenchyma during the course of its development under water ruptures all the surrounding tissue as cork does and becomes enclosed in a loose, spongy tissue the intercellular spaces of which, because they contain a large amount of air, give a white appearance to the organ. The intercellular spaces form a continuous aërating system. The tissue is in direct contact with the surrounding water. However, because it is filled with air it allows no movement of water through those passages.

Wieler (9) questions the correctness of Jost's interpretation of the phenomena of the appearance of pneumathodes. Jost maintains, as we have seen, that the formation of negatively geotropic roots and of the pneumathodes on them are direct responses to the oxygen needs of the plant. Jost made his observations on plants growing in tubs. Wieler, on the other hand, placed one-year-old plants of *Phoenix reclinata*, *Chamaerops humilis*, and *Sabal Adansoni* in nutrient solutions and kept them thus in jars. None of the plants at the beginning of the experiment showed pneumathodes. On the basis of Jost's assumption that some of the roots would grow out of the water medium and upward, negatively geotropic roots with pneumathodes would be formed. This, however, was not the case. The roots grew, like those of other plants, into the water. The roots of *Phoenix reclinata* showed many pneumathodes. The single plants of *Sabal* and *Chamaerops* produced no pneumathodes. The photographs that Wieler shows of such pneumathodes on the roots of *Phoenix* are quite similar to those shown in figure 1, Plate XXXVII, of this paper. According to Wieler, then, the formation of a pneumathode is not a reaction to an aërotropic stimulus and it is therefore not to be considered as a breathing organ. The structure of the pneumathodes produced under water agrees with the structure of those produced above water which Jost describes.



As an argument against the pneumathodes as breathing organs, Wieler states that most of the pneumathodes, being air roots, are short-lived. He states that they are of limited growth and that the growing point soon degenerates. Discarding the assumption that pneumathodes are breathing organs, Wieler assumes that the water medium stimulates increased growth at certain points of the root. This leads to a bursting of the epidermis. This wounding stimulates the plugging up of the intercellular spaces, From intumescences which were developed on the roots of plants of beech, ash, and maple grown under the same conditions as the palm plants he studied, Wieler groups the so-called pneumathodes and intumescences as results of reactions to the same medium. These intumescences have no definite function.

The observations of Wieler fit in with the phenomena listed in the various texts on plant pathology under excessive moisture conditions. Thus, Küster (5) includes, under the title of "Hyperhydrische Gewebe (a) Lenti-zellen und Rindenwucherungen," the palm described by Wieler. Cell proliferations in intumescences show the same individual structure described in the aërenchyma of the pneumathode (Küster, 5, p. 34). The loose arrangement of the aërenchyma is also duplicated in *Catalpa* (Küster, p. 54). Similar cell arrangements are found in wound and regenerative tissue (Küster, p. 63), as well as in the disease of apples called "woolly streak" (Sorauer, 8, p. 325).

Are the so-called pneumathodes found on the palms by Jost as well as those that Wieler found on *Phoenix reclinata*, Copeland on *Cocos nucifera*, and the organs found on *Elaeis guineensis* to be considered as functionless bodies resulting from the plant's reaction to a definite stimulus? It has already been pointed out that pneumathodes occur in large numbers on the adventitious roots of the oil palm, the coconut palm, and many of the other palms growing in the botanical gardens at Buitenzorg. These organs have a definite structure, and they are in direct communication with the large lacunae of the roots. The lacunae in the roots of the oil palm are very conspicuous and characteristic. Pneumathodes also occur, somewhat sparingly to be sure, on the underground roots of *Elaeis* in well aërated soil. Adventitious roots may grow for considerable distances along the surface of the soil and persist in a living condition for a long time; they become woody and rigid, they send out secondary roots, and pneumathodes are produced along their whole length. The pneumathodes appear much more abundantly on the upper surface of the prostrate root and on the outer surfaces of the roots that are growing down toward the ground. The adventitious roots that produce pneumathodes also produce secondary roots. Both originate endogenously, but their structure at maturing is quite distinct (contrast fig. 17 with figs. 19 and 20).

In the young pneumathode on the adventitious root there is a distinct aërenchyma tissue which degenerates in those pneumathodes that do not

lose their root cap and later become woody (fig. 19). This condition allows for a direct communication between the outside medium and the interior of the root.

It is true that Jost's evidence for the negatively geotropic character of the upright-growing roots bearing the pneumathodes is rather unconvincing. The induced pneumathodes on *Elaeis*, like those that Wieler found on *Phoenix reclinata*, were found in large numbers on the submerged roots and showed no tendency to be negatively geotropic.

Unfortunately, experiments on the physiological function of the pneumathodes could not be carried out. It is evident, however, that they are not organs of chance and functionless. To group them with intumescences, as do Wieler and Küster, because they show similar cellular structures to those found in hyperhydrated or callous tissue, seems to be arbitrary.

It is an interesting fact that none of the roots of the first order (fig. 1) burst their epidermis at the tip and produce the characteristic structure of the induced pneumathode.

The observations on the oil palm agree with Jost's observation that the pneumathodes in palms function as organs of gas-exchange. The observations on the root and the pneumathode of the oil palm agree with Perseke's (6) conclusions that plants grown in water and in very wet soil show intercellular spaces in their early development, and that in later growth these intercellular spaces give rise to large air spaces as a result of splitting and absorption of the cells. This, together with the formation of a hypodermis, is characteristic of hydrophyllous roots.

#### SUMMARY

Pneumathodes are produced on the adventitious roots of the oil palm.

Pneumathodes are organs of gas-exchange.

Pneumathodes originate like secondary roots but become modified secondarily.

There is a direct communication between the pneumathodes and the large air chambers in the root, allowing thus for a communication between the atmosphere and the interior of the root.

Pneumathodes show a tissue that is characteristic for these organs (modified aërenchyma).

Pneumathodes may be induced to develop on the underground roots through excessive moisture. They are then formed in large numbers.

Pneumathodes can not be considered in the same category with intumescences (Wieler and Küster) although the cells of the aërenchyma resemble the cells found in intumescences.

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## EXPLANATION OF PLATES

## PLATE XXXVII

FIG. 1. Root system of a young plant, showing the distribution of the induced pneumathodes on the youngest root. One secondary root also shows pneumathodes.

FIG. 2. Portion of a root, showing secondary roots with pneumathodes. The main root shows both secondary roots and pneumathodes.

FIG. 3. Magnified portion of a root, showing pneumathodes in various stages.

FIG. 4. Magnified portion of the end of a root, showing active formation of pneumathodes.

FIG. 5. Young pneumathode, just rupturing its epidermis.

FIGS. 6, 7. Later stages of pneumathodes, showing the mealy tissue.

FIG. 8. Greatly elongated pneumathode, showing rings on the mealy tissue and the large root cap.

FIG. 9. Portion of an adventitious root, showing large root and former places of attachment of the root cap.

FIG. 10. Portion of an adventitious root, showing the distribution of pneumathodes.

FIG. 11. Portion of an adventitious root with parts removed to show the relation of the pneumathode to the root. The pneumathode retains its root cap.

FIG. 12. Pneumathode with aërenchymous tissue exposed and partially sloughed off.

FIG. 13. Pneumathode with aërenchymous tissue exposed and more of it sloughed off.

FIG. 14. Pneumathode with aërenchymous tissue almost entirely sloughed off.

## PLATE XXXVIII

FIG. 15. Cross section through mother root and longitudinal sections through four pneumathodes. The radiating air chambers are characteristic of roots.

FIG. 16. Cross section through the base of a pneumathode, showing also a portion of the tissue of the mother root.

FIG. 17. Cross section through a secondary root formed on a mother root which showed many induced pneumathodes. A very conspicuous sclerenchymous zone is present. The characteristic air chambers appear, as well as large cells containing bundles of raphides of calcium oxalate.

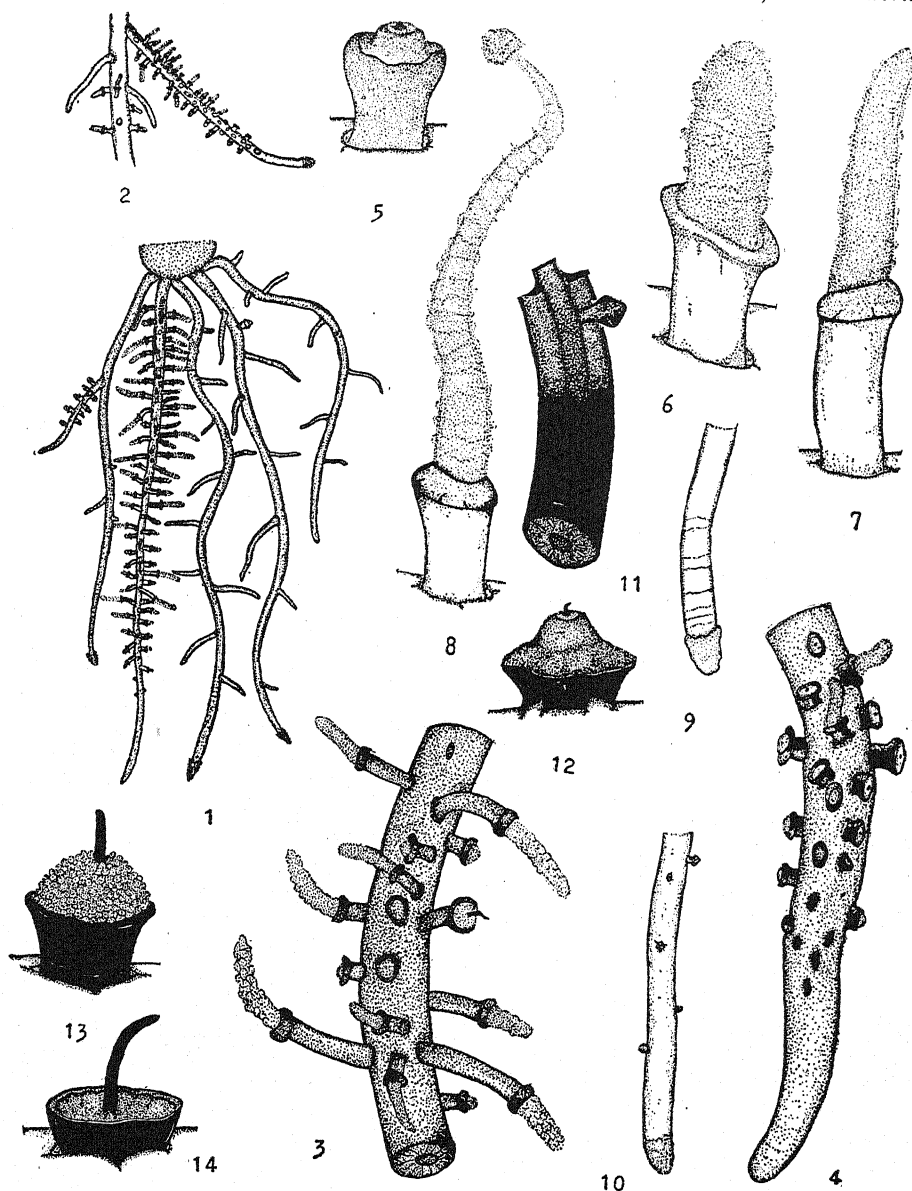
FIG. 18. Diagrammatic representation of a longitudinal section of a pneumathode on an adventitious root. The dark zones on both sides of the central vascular bundle indicate the aërenchymous tissue which has degenerated before it was sloughed off.

FIG. 19. Cross section through a pneumathode on an adventitious root. Explanation in text.

FIG. 20. Cross section through an induced pneumathode, showing the distribution of aërenchyma. This is a condition just prior to the rupturing of the epidermis.

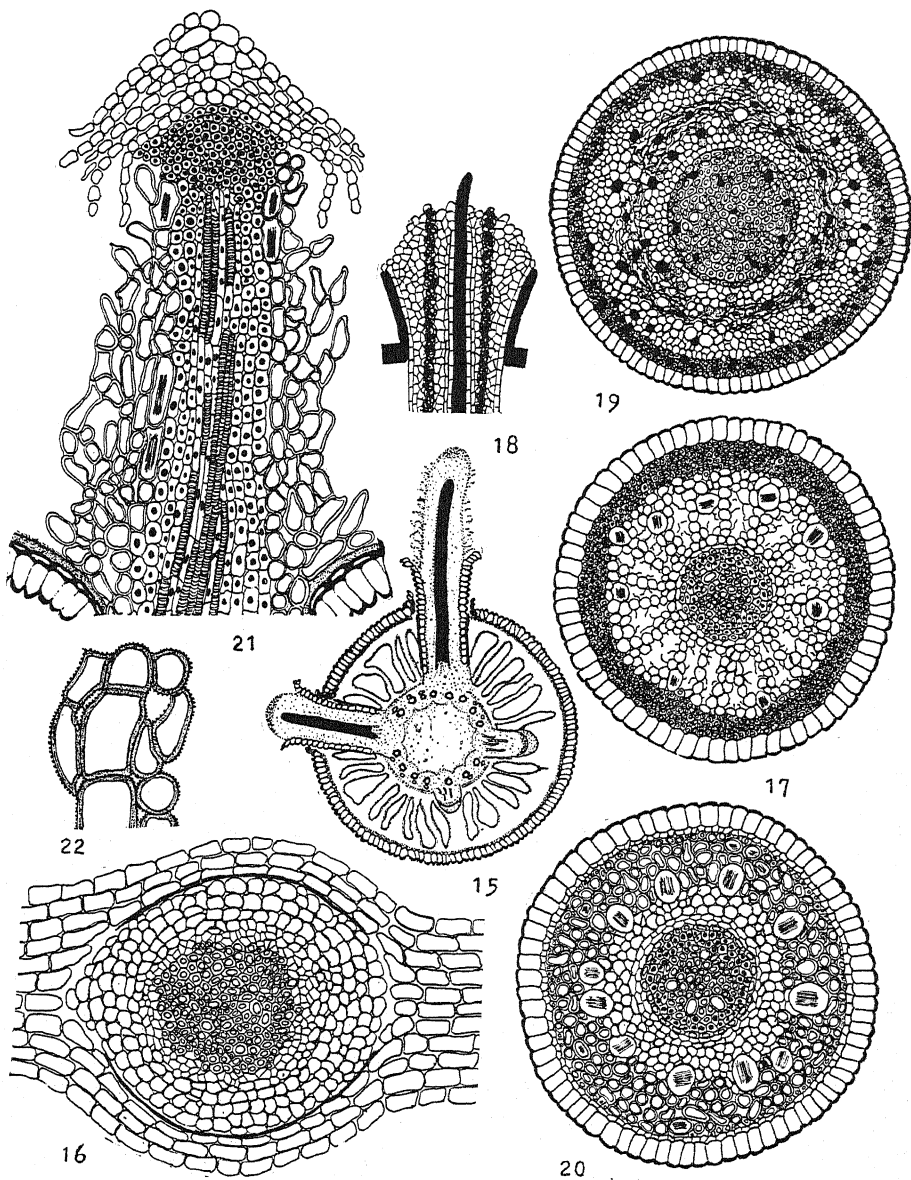
FIG. 21. Longitudinal section through an induced pneumathode. Explanation in text.

FIG. 22. Cells of aërenchyma, showing the roughened cell walls.



YAMPOLSKY: PNEUMATHODES OF THE OIL PALM





YAMPOLSKY: PNEUMATHODES OF THE OIL PALM





## THE PERSISTENCY OF *SCIRPUS VALIDUS* VAHL<sup>1</sup>

FRANK C. GATES

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Dredging a canal through the marshy ground north of Waukegan, Illinois, from Lake Michigan to the Johns-Mansville asbestos plant in 1919 has resulted in some very interesting successions from the *Scirpus validus* association. The author's first study of this area was published in a bulletin of the Illinois State Laboratory of Natural History (volume 9, article 5, 1912). At that time the *Scirpus validus* association was well developed and very characteristic of most of the swales in the western half of the southern part of the Waukegan flats (Beach area). The association was virtually a pure growth of *Scirpus validus* across many of the swales, particularly those in the western part of the flat. As one approached Lake Michigan, the swales, while they might have *Scirpus validus*, were more likely to be vegetated with the *Cladium* association.

The wide swales of *Scirpus validus* might shade out gradually or abruptly at the edges to various associations, such as the *Cladium*, *Calamagrostis*, *Salix-Cornus* thicket, or the prairie in which *Liatris spicata* (L.) Willd. was very prominent. Where sand dunes separated the swales from each other, the transition was abrupt to the *Liatris scariosa* association. The center of a few of the swales near Waukegan was open water of slight depth, bordered by such aquatic plants as water lilies or *Polygonum amphibium* L. Very few such swales had water as deep as a foot and a half, and more usually it was a matter of only two or three inches. Below the water were a few inches of colloidal mud, in the lower part of which the plants were rooted. Below occurred the sand substratum which underlies all of this area. In the *Scirpus validus* association one might occasionally find a plant of *Polygonum*, *Castalia*, or *Utricularia*, but such made up so slight an amount of the vegetation as to be practically negligible.

During 1919 a canal some 250 feet in width and 20 feet deep was dredged from Lake Michigan across the entire area to the Chicago and Northwestern railroad tracks. Previously the drainage of the area had been very slight and consisted of little more than seepage. The canal, however, provided drainage whose effect is quite obvious for nearly a half mile north. The water table was thus lowered immediately about  $2\frac{1}{2}$  to 3 feet. This resulted in complete drainage of all the open-water swales and sufficient drainage to permit a serious drying out of all the other swales for varying

<sup>1</sup> Contribution no. 208 from the Botanical Laboratory of the Kansas State Agricultural College, Manhattan, Kansas.

distances back from the canal. When seen in 1922, there was no open water in any of the swales north of the ditch. The effect upon the *Scirpus validus* association was striking within about 400 to 500 feet north of the ditch. Beyond this the effect was not particularly marked, although it must be said that there were evidences of changes noticeable to a person previously well acquainted with the area. Following the lowering of the water-table level the *Scirpus* has had a struggle to maintain itself. The roots of the plants are no longer submerged in water, and the 3 or 4 inches of turf in which they were growing now dries very nearly completely during dry years. Such was the case in 1922. It is evident that the turf is shrinking in amount, and as a result the *Scirpus* rhizomes are becoming more and more exposed on the surface. Occasional fires in some of the places have aided in the shrinking of the turf.

Under these conditions succession has been very rapid. The normal expectation under natural conditions would be for the *Scirpus validus* association to be succeeded by the *Scirpus americanus* association. This is now nowhere present, as the conditions are far too severe for *Scirpus americanus*. It has entirely disappeared from the area anywhere near the canal. The *Cladium* association might replace the *Scirpus validus*. This has taken place farther back from the canal, but is nowhere in evidence in its proximity. The *Calamagrostis* association might follow the *Scirpus validus* directly, or might follow the *Cladium* which has followed the *Scirpus validus*. Conditions for *Calamagrostis*, however, have been so favorable, and this succession has taken place so rapidly, that over considerable areas the *Cladium* association has been supplanted before it had time to supplant the *Scirpus validus*. Such areas now show as *Calamagrostis* in which there may be an occasional plant of *Scirpus validus* still persisting as a relic. In other cases it is possible for the *Liatris spicata* prairie to invade the *Scirpus validus* association directly or through the above-mentioned intermediate stages. Conditions have favored this succession, so that now in places prairie exists where only recently *Scirpus validus* was present. Under such conditions *Solidago graminifolia* (L.) Salisb. is the species most frequently and most abundantly represented. The prairie grasses are somewhat tardy in making their appearance—the grasses that occur being for the most part wet-ground grasses, such as species of *Muhlenbergia* or *Panicum*. These are not so tall as *Scirpus validus* and do not have the ability to drive it out. Consequently the two are growing on the same area of ground, although it is obvious that *Scirpus validus* is gradually losing out. In other cases, the *Scirpus* has thinned somewhat and an abundance of secondary growth is present (text fig. 1). This secondary growth seldom exceeds about one third of the height of the *Scirpus* culms. Composing it for the most part are the land form of *Polygonum amphibium* L., persisting from the time when open water was present, *Mariscus mariscoides* (Muhl.) Kuntze, and *Epilobium coloratum* Muhl. When grasses occur

they are usually species of *Muhlenbergia* or *Panicum*. Occasionally *Spartina michauxiana* Hitchc. is present, although nowhere in the abundance that one might expect.

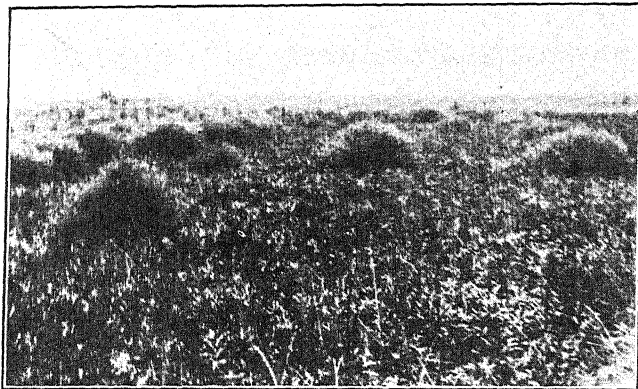


TEXT FIG. 1. A former *Scirpus* area, now a mixture of vegetation in which *Erigeron canadensis* and *Muhlenbergia* sp. are most abundant, although many *Scirpus* culms remain. August 27, 1923.

For the most part, shrubs and trees are very slow to appear. Perhaps the occasional fires are to be considered responsible for this. When shrubs appear, species of *Salix* or *Cornus* are usually the typical ones. No trees were noted in any of the marshy part of the area, however, although two small seedlings of *Populus deltoides* Marsh were found.

The conditions which have been described so far are those out of the immediate proximity of the canal. In its immediate proximity, the changes have been very much more striking and decidedly out of the ordinary. Here one finds the swales literally covered with certain types of weeds. These vary somewhat from year to year, but still in no case have they entirely eliminated *Scirpus validus* from areas in which it formerly dominated. During 1922, *Erigeron canadensis* L. was by far the commonest weed in these areas. It made a very curious impression to see this upland weed growing very densely over an area in which there were a few *Scirpus* culms projecting above them. In 1923 this familiar weed was quite common, although there were others that had assumed dominance for that season. Among these, *Salsola pestifer* A. Nelson was most prominent. This weed, the familiar Russian thistle, was formerly in the area on the ballast of the Elgin, Joliet, and Eastern railroad at the extreme southern part of the area. Not very much of it was noticed in 1922, but in 1923 it was quite common along the north bank of the canal and over the deepest swales. Here, where formerly open water had been, a low growth of the land form of *Polygonum amphibium* is likely to be present, in which there are large bushy plants of

*Salsola*, both where the open water has been and in the areas where *Scirpus validus* was abundant. Growing in the former place, the bushes of *Salsola* might occasionally be 4 feet in diameter (text fig. 2), but when growing in the *Scirpus* the plants were more crowded and, in consequence, somewhat higher and less bushy. Like *Erigeron*, *Salsola* apparently does not have



TEXT FIG. 2. A former swale with open water, now largely covered with the land form of *Polygonum amphibium* and large "bushes" of *Salsola pestifer*. Occasional culms of *Scirpus validus* remain. August 27, 1923.

the ability to eliminate *Scirpus*, as the *Scirpus* culms were growing vigorously up through it. Adjacent to these swales on some of the old sand ridges, the prairie was represented by *Liatris cylindracea* Michx., sometimes forming a dense growth, at other times a rather open growth. It was quite obvious that bushes of *Salsola* had tumbled over these ridges, as some were retained upon them, yet no seeds grew even in the most open places.

In the centers of some of the deeper swales which had not been completely covered by *Scirpus*, the weed element was especially well developed and was spreading into the *Scirpus* on either side. In some such places *Calamagrostis canadensis* (Michx.) Beauv. occurred along with the weeds and gave some evidences of developing a meadow in advance of its usual position in the normal genetic series. Frequently in such places *Scirpus validus* showed a spiral growth—evidence of an unfavorable habitat. Other weeds frequently present included particularly *Amaranthus retroflexus* L., *Lactuca canadensis* L., and *Panicum capillare* L. These might be mixed with some normal prairie and wet-ground plants such as *Eupatorium perfoliatum* L., *Koelia virginiana* (L.) MacM., *Hypericum virginicum* L., *Lycopus americanus* Muhl., *Aster ptarmicoides* T. & G., *Iris versicolor* L., and *Typha latifolia* L., in addition to those which have been mentioned in the earlier part of the paper.

Just how long the *Scirpus* will be able to maintain itself is a question of some interest, for the plants which have so far come into the *Scirpus* area

do not appear to be eliminating it very rapidly. In fact, in many cases they seem to be staving off the final outcome because, by their own shade, the ground is prevented from drying out so thoroughly. The places in which the *Scirpus* is now entirely gone are those which have been exposed to the sun, or close to the canal where tramping has kept down all vegetation, and a few small areas where fires have been built. Under these conditions the turf has been broken up and blown away. The exposed sand is essentially plantless.

#### SUMMARY

1. Thorough drainage of swales of *Scirpus validus* Vahl resulted in greatly accelerating normal succession to land-plant associations, in which some plants of *Scirpus validus* remained as relics.
2. Thorough drainage accompanied by disturbance of the soil resulted in an influx of certain common weeds, particularly *Erigeron canadensis* L. and *Salsola pestifer* A. Nelson, both in areas formerly open water, and in areas dominated by *Scirpus validus*.
3. At least four years of living under dry-land conditions has not been sufficient in itself to eliminate plants of *Scirpus validus*.

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE INFECTION  
OF SORGHUMS AND OATS BY SMUTS I. EXPERI-  
MENTS WITH COVERED AND LOOSE KERNEL  
SMUTS OF SORGHUM<sup>1</sup>

GEORGE M. REED AND JAMES A. FARIS

(Received for publication December 10, 1923)

The influence of environmental conditions on the prevalence of cereal smuts has been recognized for many years. Numerous observers have recorded a possible connection between the weather and the occurrence of smut in the crop. A few investigators have analyzed the relation and emphasized the probable rôle of soil temperature and moisture during the germination period of the host.

The experiments here described were undertaken to determine the relation of some soil factors to the infection of sorghums by *Sphacelotheca sorghi* (Link) Clint. and *S. cruenta* (Kühn) Potter, and of oats by *Ustilago levis* (K. & S.) Magn. The factors studied were the temperature, moisture, and reaction of the soil during the period of seed-germination. The investigations were begun in 1922, and the methods in the early experiments were varied in order to determine the procedure best adapted for the studies undertaken.

METHODS

In practically all the experiments with temperature, the plants were grown in specially constructed tanks which have been described by the junior author.<sup>2</sup> These were heated and accurately controlled by electricity, and the temperatures used were very constant.

In some of the experiments the seeds were planted in soil which had been sifted and made as uniform as possible. This soil gave a neutral reaction. It was found, however, that sand served the purposes of the experiments very satisfactorily, and for the later work it was used entirely. The sand was a good grade of builders' sand, thoroughly washed and graded to pass through a 20-mesh but not through a 40-mesh sieve. The water content and the water-holding capacity of the soil or sand were accurately determined. Weighed quantities were taken for each experiment, and the required amount of water was added and thoroughly mixed. It was very

<sup>1</sup> Brooklyn Botanic Garden Contributions no. 36. The writers desire to record their appreciation of the valuable assistance rendered by Miss Lois E. Davis in carrying out the experiments and in preparing the data for publication.

<sup>2</sup> Faris, J. A. Factors influencing infection of *Hordeum sativum* by *Ustilago hordei*. Amer. Jour. Bot. 11: 189-214. 1924.



much easier to obtain uniformity of moisture content with the sand than with the soil, and this consideration very largely led to the use of sand in the experiments.

The spores of the different smuts were collected in the experimental plot of the previous season and preserved. The smutted panicles were ground up, and the spores were freed from the *débris* by sifting. The seed for inoculation was placed in a small envelope and a sufficient quantity of spores added to dust it thoroughly.

The sand or soil after being prepared was placed in paraffined paper cups, filling them about half full, and tamped. The required number of seeds were then spread over the surface and covered to a uniform depth of one inch by sand or soil of the same character. The cups were then placed in closed glass chambers and suspended in the temperature tanks.

For many of the experiments on the influence of soil moisture, cultures were prepared in the same fashion in the paraffined cups but were grown at room temperature. In these cases there was a slight variation of temperature, but on the average it was  $21^{\circ}$  C. At no time did it vary below  $19^{\circ}$  or above  $22^{\circ}$  C.

Accurate counts were kept of the germination of the seeds under the various conditions, the counts being made as soon as the seedlings emerged from the soil. After the plants had emerged from the soil or sand they were removed from the temperature tank, and as soon as possible were transplanted to the field. In general, no special difficulty was experienced in the operation of transplanting.

#### RESULTS IN 1922

*Temperature Series.* During 1922, experiments were carried out with the covered smut, *Sphacelotheca sorghi*, several varieties of sorghums being used in connection with the different experiments. Four susceptible varieties, Valley Kaoliang (192), Shallu (196), Blackhull Kafir (223), and Red Amber Sorgo (170), were germinated in sand with a moisture content of 40 percent and a soil reaction of pH 7.2 at a number of different temperatures in the constant-temperature tanks. The detailed results are presented in table I and illustrated in the graph of text figure 1.

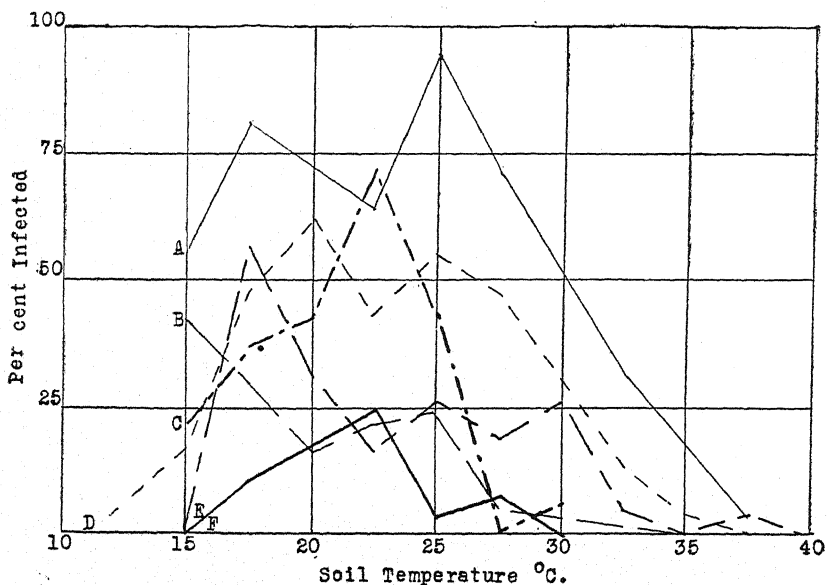
Valley Kaoliang (192) was germinated at temperatures varying from  $12^{\circ}$  to  $39\frac{1}{2}^{\circ}$  C. The highest infection (62.5 percent) occurred at  $20^{\circ}$ . However, quite high percentages of infection were obtained at all temperatures between  $17\frac{1}{2}^{\circ}$  and  $30^{\circ}$ . At  $12^{\circ}$  and  $15^{\circ}$ , as well as at temperatures of  $32\frac{1}{2}^{\circ}$  and above, there was a decided decrease in the percentages of infection, negative results being obtained at temperatures of  $37\frac{1}{2}^{\circ}$  and  $39\frac{1}{2}^{\circ}$ .

Shallu (196) was grown at temperatures between  $15^{\circ}$  and  $37\frac{1}{2}^{\circ}$  C.; the highest infection (93.8 percent) was obtained at a temperature of  $25^{\circ}$ , and high infections occurred at all temperatures between  $15^{\circ}$  and  $32\frac{1}{2}^{\circ}$ . There was a marked decrease in infection at  $37\frac{1}{2}^{\circ}$ .

TABLE I. Influence of Temperature on Infection of Susceptible Sorghums by *Sphacelotheca sorghi* (Link) Clint., 1922

(Sand, 40 percent moisture; pH 7.2)

Temperature	Tank Series								Incubator Series			
	Valley Kaoliang (192)		Shallu (196)		Blackhull Kafir (223)		Red Amber Sorgo (170)		Manchu Kaoliang (191)		Sumac Sorgo (171)	
	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
10-12° C....	25	4.0	—	—	18	0	—	—	—	—	—	—
15.....	12	16.6	9	55.5	19	0	10	0	58	22.4	14	42.8
17.5.....	21	47.6	30	80.0	25	56.0	19	10.5	16	37.5	10	30.0
20.....	80	62.5	—	—	74	31.0	—	—	37	43.2	19	15.7
22.5.....	23	43.4	17	64.7	25	16.0	12	25.0	7	71.4	14	21.4
25.....	83	55.4	49	93.8	86	26.7	26	3.8	86	38.3	57	22.8
27.5.....	40	47.5	42	71.4	37	18.9	26	7.6	27	0	20	5.0
30.....	35	31.4	39	51.2	38	26.3	19	0	33	6.0	28	3.5
32.5.....	41	12.1	41	31.7	35	5.7	21	0	—	—	—	—
35.....	34	2.9	—	—	29	0	—	—	—	—	22	0
37.5.....	29	0	41	2.4	34	2.9	29	0	—	—	—	—
39.5.....	31	0	—	—	20	0	—	—	—	—	—	—

TEXT FIG. 1. Influence of temperature on infection of sorghums by *Sphacelotheca sorghi*, 1922. A, Shallu. B, Sumac Sorgo. C, Manchu Kaoliang. D, Valley Kaoliang. E, Blackhull Kafir. F, Red Amber Sorgo.

Blackhull Kafir (223) was grown at temperatures between 12° and 39½°. C. No infection occurred at the two lower temperatures of 12° and 15°; the highest infection (56 percent) occurred at 17½°. There was then a

decrease, but a still relatively high percentage of infection at temperatures ranging up to 30°. Beyond that point the percentages of infection were small, or the results were negative.

Red Amber Sorgo (170) was grown at temperatures between 15° and 37½° C. No infection occurred at 15° or above 27½°; the highest percentage of infection (25 percent) occurred at 22½°.

Two additional susceptible varieties of sorghum, Manchu Kaoliang (191) and Sumac Sorgo (171), also were grown in sand with a moisture content of 40 percent and a pH concentration of 7.2. These varieties, however, were germinated in incubators instead of in the constant-temperature tanks. The temperatures were not as accurately controlled, and there was the further disadvantage that germination took place in complete darkness; otherwise the treatment was the same.

Manchu Kaoliang (191) was germinated at temperatures from 15° to 30°C. The highest percentage of infection (71.4 percent) was obtained at 22½°. Relatively high percentages of infection were obtained, however, at temperatures from 15° to 25°. Above 25° there was a marked decrease, or even negative results.

TABLE 2. *Influence of Moisture and Temperature on Infection of Blackhull Kafir and Red Amber Sorgo by Sphacelotheca sorghi (Link) Clint., 1922*  
(Soil, pH 7; tank series)

*Blackhull Kafir (223)*

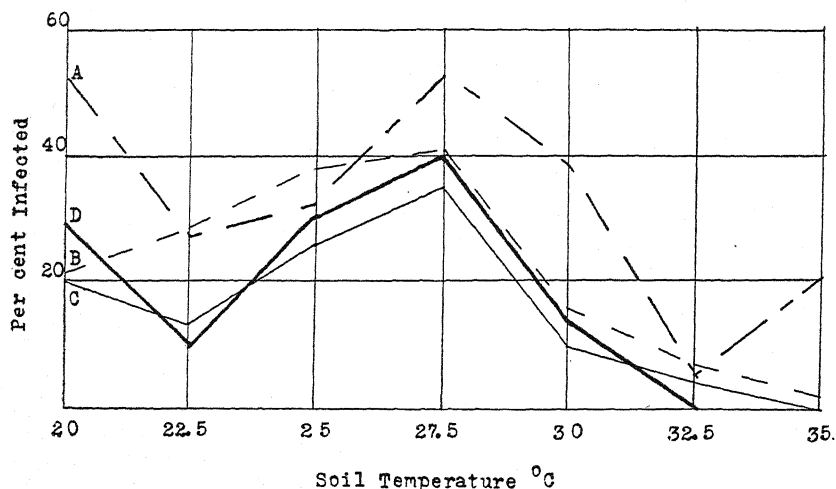
Temperature	47 percent Moisture		60 percent Moisture		70 percent Moisture		80 percent Moisture	
	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
20.0° C.....	23	52.1	24	20.8	10	20.0	17	29.4
22.5.....	36	27.7	53	28.3	44	13.6	28	10.7
25.0.....	27	33.3	29	37.9	23	26.0	23	34.7
27.5.....	21	52.3	32	40.6	28	35.7	29	37.9
30.0.....	36	38.8	31	16.1	30	10.0	29	13.7
32.5.....	18	5.5	27	7.4	20	5.0	20	0
35.0.....	29	20.6	42	2.3	46	0	34	0

*Red Amber Sorgo (232)*

Temperature	47 percent Moisture		60 percent Moisture		70 percent Moisture		80 percent Moisture	
	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
20.0° C.....	34	64.7	28	53.5	29	20.6	21	9.5
22.5.....	59	52.5	81	41.9	63	23.8	29	13.7
25.0.....	32	65.6	33	57.5	31	29.0	20	10.0
27.5.....	23	56.5	31	58.0	31	61.2	25	44.0
30.0.....	35	62.8	39	51.2	36	5.5	29	3.4
32.5.....	12	33.3	13	0	21	4.7	12	0
35.0.....	36	8.3	42	0	38	0	28	0

Sumac Sorgo (171) was grown at temperatures between 15° and 35° C. The highest percentage of infection (42.8 percent) was obtained at 15°, with relatively high percentages at temperatures up to 25°. Beyond that temperature there was a decrease in the number of plants infected.

In the experiments with all these six varieties, there is some irregularity in the curves which show the relation of infection to temperature. It is clearly established, however, that relatively high percentages of infection occur over a wide range of temperatures. Below 15° C. and above 30° C. there is a decrease in the amount of infection, which, however, is somewhat dependent upon the variety.



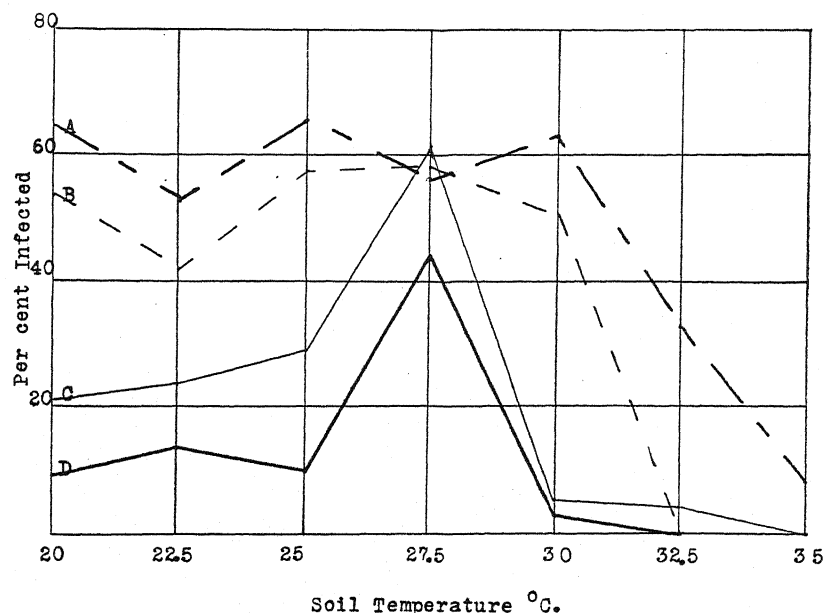
TEXT FIG. 2. Influence of soil moisture and temperature on infection of Blackhull Kafir by *Sphacelotheca sorghi*, 1922. A, 47 percent moisture. B, 60 percent moisture. C, 70 percent moisture. D, 80 percent moisture.

**Moisture Series.** Blackhull Kafir (223) and Red Amber Sorgo (232) were germinated in soil testing pH 7 to which different amounts of water were added, four moistures—47, 60, 70, and 80 percent—being used. The cultures were germinated at a number of different temperatures varying from 20° to 35° C. The results are presented in table 2 and illustrated in the graphs of text figures 2 and 3.

While there was considerable irregularity in the results obtained, yet certain facts stand out quite clearly. With Blackhull Kafir (text fig. 2), in general the highest percentages of infection were obtained in a soil moisture of 47 percent, and there was no striking difference at any of the temperatures from 20° to 30° C; at the two higher temperatures, however, there was a decrease in the amount of infection. In the 60-percent moisture, the percentages of infection were generally lower than those at 47 percent, but the range for rather high percentages of infection was the same. In the

70-percent moisture, the percentages of infection were lower than those in 60-percent moisture, relatively high percentages of infection being obtained at temperatures between 20° and 30°. In the 80-percent moisture, the percentages of infection were about the same, perhaps slightly higher than in the 70-percent moisture.

In every case the highest percentage of infection was obtained at 27½° C., and the range for moderately high infections lay from 20° to 30°. At the higher temperatures of 32½° and 35° the results were either low percentages of infection or entirely negative. Unfortunately, temperatures below 20° were not included in this series.



TEXT FIG. 3. Influence of soil moisture and temperature on infection of Red Amber Sorgho by *Sphacelotheca sorghi*, 1922. A, 47 percent moisture. B, 60 percent moisture. C, 70 percent moisture. D, 80 percent moisture.

Similar results were secured with Red Amber Sorgho (text fig. 3) with, in general, higher percentages of infection. The highest percentages were obtained in the 47-percent moisture and extended over the temperature range from 20° to 30° C. In 60-percent moisture, the percentage of infection was, with one exception, lower than in 47-percent moisture, with high infections at all temperatures between 20° and 30°. In the 70-percent moisture, rather low percentages of infection were obtained, except at the temperature of 27½°. The curve of infection in the 80-percent moisture is very similar to that in the 70-percent with, however, in every case a lower percentage of infection. The highest percentage of infection in the

47-percent moisture was obtained at 25°; while in the other three moistures the highest percentages were obtained at 27½°.

*Results with Resistant Varieties.* In addition to these highly susceptible varieties of sorghums, a number of experiments were carried out with certain varieties which possess a well marked resistance to *Sphacelotheca sorghi*. The results secured with these varieties are given in table 3.

TABLE 3. *Influence of Temperature on Infection of Resistant Sorghums by Sphacelotheca sorghi, 1922*

(Sand, 40 percent moisture; pH 7.2)

Variety	Seed No.	No. Constant Temp. Used	Temperature Ranges	No. Plants	No. Infected
Darso.....	225	7	15 -30° C.	183	0
Feterita.....	182	10	12 -39.5	447	1
Feterita.....	226	7	17.5-37.5	204	0
Milo, Dwarf Yellow.....	224	11	12 -39.5	384	0
Milo, Standard Yellow.....	194	6	17.5-30.0	301	0
Milo, White.....	195	9	15 -37.5	386	0

Although Darso (225), Feterita (226), Dwarf Yellow Milo (224), Standard Yellow Milo (194), and White Milo (195) were grown at several different constant temperatures over fairly wide temperature ranges, not a single infection occurred. Feterita (182), grown at ten different constant temperatures over a temperature range of 12° to 39½° C., gave one infected plant out of a total of 447. In other experiments of the senior author, an occasional infected plant of Feterita has been noted. The other varieties, however, have consistently given negative results. The results clearly indicate that these varieties maintain their high degree of resistance to infection by *Sphacelotheca sorghi* over a wide range of temperature.

#### RESULTS IN 1923

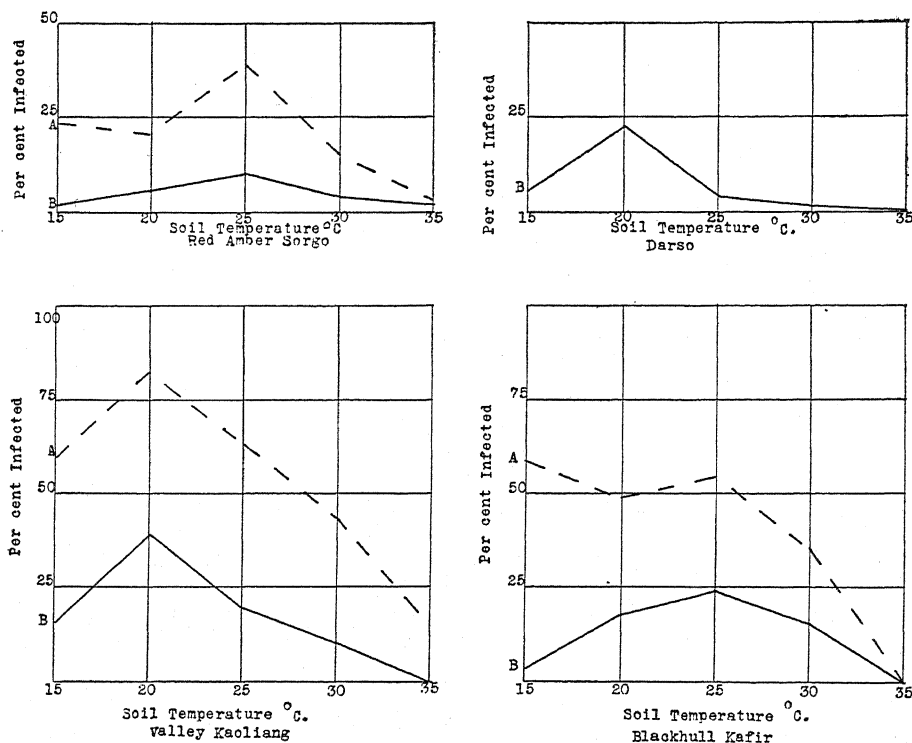
The methods used in 1923 were essentially the same as those described above. The experiments were carried out with both the covered smut, *Sphacelotheca sorghi*, and the loose smut, *S. cruenta*. Precautions were taken in order to prevent the mixing of the spores of the two species. This also involved special care in inoculating the seed.

Four different varieties of sorghums were used: Darso (225), Valley Kaoliang (192), Blackhull Kafir (223), and Red Amber Sorgo (232). Darso is particularly interesting because previous experiments by the senior author have shown that it is moderately susceptible to *Sphacelotheca cruenta*, but, on the other hand, no infections by *S. sorghi* have been noted on this variety. So far as known at present, it is the only variety of sorghum which is susceptible to one of the smuts and highly resistant to the other. All the other sorghums studied, if resistant to one smut are also resistant to the other, and if susceptible to one smut are more or less susceptible to the other.



The remaining three varieties—Valley Kaoliang, Blackhull Kafir, and Red Amber Sorgho—have shown themselves to be susceptible to both smuts. They, however, have given higher percentages of infection by the covered smut than by the loose smut.

*Temperature Series with Sphacelotheca sorghi.* All four varieties of sorghums were employed in the temperature series with *Sphacelotheca sorghi*. They were germinated in sand with a soil moisture of 30 percent and a soil



TEXT FIG. 4. Influence of temperature on infection of sorghums by *Sphacelotheca sorghi* and *S. cruenta*, 1923. A, *Sphacelotheca sorghi*. B, *Sphacelotheca cruenta*.

reaction of pH 7.2, and kept in the constant-temperature tank at the desired temperature until the seedlings had emerged from the surface of the sand. The experiments were carried out at temperatures of 15°, 20°, 25°, 30°, and 35° C. The results obtained are recorded in table 4 and illustrated in the graph of text figure 4.

Darso (225), which, as previously mentioned, has shown itself to be highly resistant to covered kernel smut, remained entirely free from infection at all of the five different temperatures, a total of 318 plants being grown.



TABLE 4. Influence of Temperature on Infection of Sorghums by *Sphacelotheca sorghi* and *S. cruenta*  
(Sand, 30 percent moisture; pH 7.2)  
*Sphacelotheca sorghi* (Link) Clint.

Variety	Seed No.	15° C.		20° C.		25° C.		30° C.		35° C.		Variable Temperature	
		No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
Darso .....	225	47	0	46	0	82	0	61	0	82	0	48	0
Kafir, Blackhull .....	223	47	59.5	63	49.2	77	53.2	77	35.0	59	1.6	77	50.6
Kaoliang, Valley .....	192	92	58.6	90	77.7	93	63.4	90	42.2	55	14.5	84	48.8
Sorgo, Red Amber .....	232*	69	23.1	62	20.9	95	37.8	96	14.5	90	2.2	94	22.3
Sorgo, Red Amber .....	232†			61	18.0	90	30.0	86	13.9	100	3.0		
Sorgo, Red Amber .....	232†			135	17.7	94	23.4	64	20.3	100	2.0		

*Sphacelotheca cruenta* (Kühn) Potter

Variety	Seed No.	15° C.		20° C.		25° C.		30° C.		35° C.		Variable Temperature	
		No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
Darso .....	225	39	7.6	53	24.5	22	4.5	72	1.3	82	0	47	6.3
Kafir, Blackhull .....	223	29	3.4	41	17.0	22	22.7	59	15.2	70	1.4	53	18.8
Kaoliang, Valley .....	192	67	14.9	64	40.6	107	17.7	101	10.8	68	0	71	18.8
Sorgo, Red Amber .....	232	62	1.6	43	4.6	108	9.2	90	3.3	109	0.9	58	5.1

\* 20 percent moisture.

† 40 percent moisture.

Valley Kaoliang (192) was infected at all the five temperatures, the highest infection (77.7 percent) occurring in the seedlings germinated at 20° C. There were also rather high infections at 15° (58.6 percent), 25° (63.4 percent), and 30° (42.2 percent). The percentage of infection at 35° was 14.5 percent; probably the maximum temperature for infection does not lie much beyond this point.

Blackhull Kafir (223) gave the highest percentage of infection (59.5 percent) at 15° C. High percentages also were obtained at 20° (49.2 percent), 25° (53.2 percent), and 30° (35 percent). Only 1.6 percent of the plants were infected at 35°.

Red Amber Sorgo (232) gave the highest percentage of infection (37.8 percent) at 25° C. The number of plants infected at 15° (23.1 percent) was slightly greater than at 20° (20.9 percent); only 14.5 percent of the plants were infected at 30°, and 2.2 percent at 35°.

In addition to these cultures of Red Amber Sorgo in sand with 30-percent moisture, this variety was also grown in sand moistures of 20 percent and 40 percent at temperatures of 20°, 25°, 30°, and 35° C. There was very little difference in the percentages of infection at 20° in sand moistures of 20, 30, and 40 percent, although the highest (20.9 percent) was obtained in the 30-percent moisture. There was a somewhat greater difference in the percentage of infection at 25°, the highest (37.8 percent) being obtained in the 30-percent moisture and the lowest (23.4 percent) in the 40-percent moisture. The highest percentage of infection (20.3 percent) was obtained in the 40-percent moisture at 30°. Very low percentages of infection were secured at 35° in all moistures.

It will be noted that infections in these three varieties of sorghum occurred over a very wide temperature range. The temperature for highest infection with Blackhull Kafir was 15° C., for Red Amber Sorgo 25°, and for Valley Kaoliang 20°. The minimum temperature for infection was not determined because a sufficiently low temperature was not used. In 1922, however, difficulty had been encountered in getting sorghums to germinate at temperatures around 12°. A temperature of 35° lies very close to the maximum temperature for infection in the varieties studied.

An experiment was carried out in which the tank was kept at a temperature of 15° C. for the first four days of germination. The temperature was raised to 35° during the next 24 hours, and then was permitted to drop to 15°, being kept at the latter temperature until the seedlings had emerged. As may be seen from table 4, the results with these three susceptible varieties were slightly lower than at the constant temperature of 15°.

*Temperature Series with Sphacelotheca cruenta.* A parallel series of experiments was carried out in which the seed of the four sorghums was inoculated with spores of *Sphacelotheca cruenta*. The methods in every respect were similar to those employed with *S. sorghi*. The results also are given in table 4 and illustrated in the graph of text figure 4.

Darso (225) gave the highest infection (24.5 percent) at a temperature of 20° C. Comparatively low percentages of infection were obtained at the other temperatures, the results being negative at 35°. Blackhull Kafir (223) gave the highest infection (22.7 percent) at 25° C.; fairly high infections were obtained at both 20° and 30°, but there was a marked decrease at 15° and 35°. Valley Kaoliang (192) gave the highest infection (40.6 percent) at 20°; moderate infections were obtained at 20° and 25°, the results at 30° being low and at 35° negative. Red Amber Sorgo (232) throughout gave low percentages of infection; the highest (9.2 percent) was obtained at 25°.

In sand with a moisture of 30 percent at pH 7.2, two of the varieties, Darso and Valley Kaoliang, gave the highest infections at 20° C., and the other two, Blackhull Kafir and Red Amber Sorgo, gave their highest infections at 25°.

Seedlings of all four varieties were grown in an experiment in which the temperature for the first four days was kept at 15° C., then raised to 35° for one day, and afterward lowered to 15° at which the plants were kept until germination was completed. The percentages of infection were somewhat higher, except with Darso, than in the experiment with constant temperature at 15° C.

*Moisture Series with Sphacelotheca sorghi.* In the moisture series, experiments were carried out using sand with moisture contents of 10, 20, 30, 40, 50, 60, 70, 80, and 90 percent. The seedlings were all germinated in the laboratory, where the temperature was approximately 21° C., but varied between 19° and 22° during the germination period. The results with *Sphacelotheca sorghi* are shown in table 5 and illustrated in text figure 5.

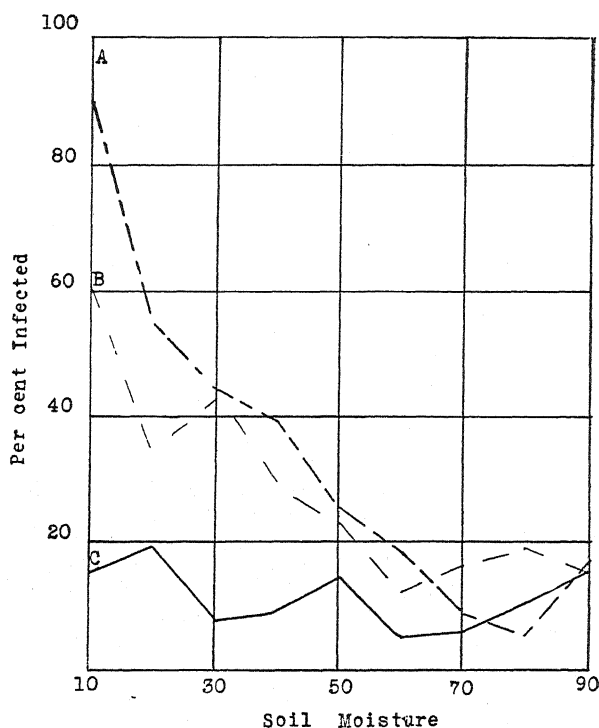
TABLE 5. *Influence of Moisture on Infection of Sorghums by Sphacelotheca sorghi, 1923*  
(Laboratory temperature, 19°–22° C. Sand, pH 7.2)

Moisture Percent	Darso (225)		Blackhull Kafir (223)		Valley Kaoliang (192)		Red Amber Sorgo (232)	
	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
10.....	61	0	91	90.1	28	60.7	87	14.9
20.....	82	0	84	54.7	106	36.7	102	19.6
30.....	37	0	73	45.2	66	43.9	87	8.0
40.....	68	0	86	39.5	86	25.5	58	8.6
50.....	50	0	84	25.0	72	22.2	107	14.0
60.....	76	0	96	19.7	116	12.0	94	7.4
70.....	23	0	68	8.8	99	16.1	95	6.3
80.....	37	0	46	6.5	68	19.1	72	11.1
90.....	28	0	72	16.6	68	14.7	51	0

Darso (225) gave entirely negative results although 462 plants were grown in the eight different moistures. Blackhull Kafir (223) gave 90.1 percent infection in the 10-percent moisture. There was a fairly uniform

but rapid decrease in the percentages of infection at the higher moistures. The only irregularity appeared in the experiment in which 90-percent moisture was used. In that case there was an increase in the number of plants infected as compared with the 70- and 80-percent moistures.

Valley Kaoliang (192) gave somewhat similar results; the highest infection (60.7 percent) was obtained in the 10-percent moisture. Fairly high percentages of infection were also obtained at moistures of 20, 30, and 40 percent. At the higher moistures the infections were generally somewhat greater than with Blackhull Kafir.



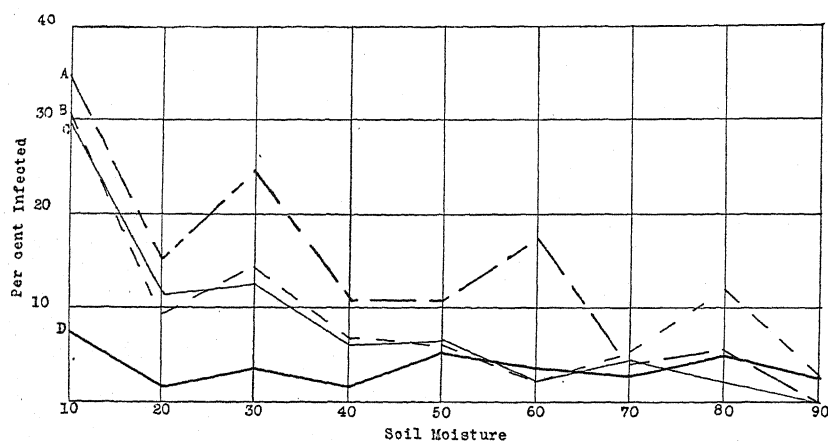
TEXT FIG. 5. Influence of soil moisture on infection of sorghums by *Sphacelotheca sorghi*, 1923. A, Blackhull Kafir. B, Valley Kaoliang. C, Red Amber Sorgo.

Red Amber Sorgo (232) in general gave rather low percentages of infection, and the results are much more irregular with this variety than with the other two. The highest percentage (19.6 percent) was obtained in the 20-percent moisture.

*Moisture Series with Sphacelotheca cruenta.* The same four varieties were inoculated with spores of *Sphacelotheca cruenta* and grown in a similar moisture series. The results are given in table 6 and illustrated in text figure 6.

TABLE 6. *Influence of Moisture on Infection of Sorghums by Sphacelotheca cruenta*, 1923  
(Laboratory temperature, 19°-22° C. Sand, pH 7.2)

Moisture Percent	Darso (225)		Blackhull Kafir (223)		Valley Kaoliang (192)		Red Amber Sorgo (232)	
	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
10.....	47	29.7	49	34.6	62	30.6	79	7.7
20.....	61	11.4	65	15.3	86	9.3	108	1.8
30.....	57	12.2	82	24.3	70	14.2	81	3.7
40.....	49	6.1	91	10.9	105	6.6	110	1.8
50.....	61	6.5	83	10.8	98	6.1	99	5.0
60.....	40	2.5	63	17.4	83	2.4	86	3.4
70.....	48	4.1	75	4.0	97	5.1	73	2.7
80.....	47	2.1	84	5.9	83	12.0	54	5.5
90.....	46	0	71	0	60	3.3	65	3.0

TEXT FIG. 6. Influence of soil moisture on infection of sorghums by *Sphacelotheca cruenta*, 1923. A, Blackhull Kafir. B, Valley Kaoliang. C, Darso. D, Red Amber Sorgo.

Darso (225) gave the highest infection (29.7 percent) in the 10-percent moisture. There was then a fairly uniform decrease in the amount of smut as the moisture increased. A similar relation holds for Blackhull Kafir (223), the highest percentage of infection (34.6 percent) occurring at the 10-percent moisture. Some irregularities occurred in the curve showing the infections in the different amounts of moisture. Valley Kaoliang (192) also gave similar results, the highest infection (30.6 percent) being obtained in the 10-percent moisture. Red Amber Sorgo (232) gave comparatively low infections, but the highest (7.7 percent) also was obtained in the 10-percent moisture.

The records indicate quite clearly that the highest infections are obtained in the low soil moistures. This is true of all four varieties. It is interesting also to note that the varieties have given similar results with

both smuts; in general, however, much higher percentages of infection were obtained with the covered than with the loose smut.

*Influence of Soil Reaction on Infection of Sorghums by Sorghum Smuts.* A series of experiments was carried out in 1922 on the influence of hydrogen-ion concentration on infection. Two varieties of sorghum, Blackhull Kafir (223) and Red Amber Sorgo (232), were inoculated with the spores of *Sphacelotheca sorghi*. The seeds were germinated in sand with 40 percent moisture and at laboratory temperature (19°–22° C.) The reaction of the sand was varied by the addition of definite quantities of very dilute sulfuric acid or potassium hydroxid, and, after the addition of the acid or alkali, the reaction of the sand was determined by the colorimetric method. The pH values actually employed were 5.2, 5.4, 6.0, 6.2, 6.6, 7.2, 7.6, and 8.4. In addition to the sand cultures, two sets were run in soil with pH reactions of 6.2 and 6.4. The results are shown in table 7 and illustrated in text figure 7.

TABLE 7. *Influence of Acidity on Infection of Blackhull Kafir and Red Amber Sorgo by Sphacelotheca sorghi, 1922*

(Laboratory temperature, 19°–21° C. Sand moisture, 40 percent)

pH	Blackhull Kafir (223)		Red Amber Sorgo (232)	
	No. Plants	Percent Inf.	No. Plants	Percent Inf.
5.2.....	19	0	22	0
5.4.....	31	3.2	25	8.0
6.0.....	31	35.4	29	10.3
6.2.....	31	41.9	21	38.0
6.6.....	33	30.3	30	6.6
7.2.....	37	21.6	31	12.9
7.6.....	31	9.6	28	7.1
8.4.....	24	4.1	18	0
*6.2.....	15	40.0	*17	29.4
*6.4.....	16	56.2	*32	37.5

\* Soil check.

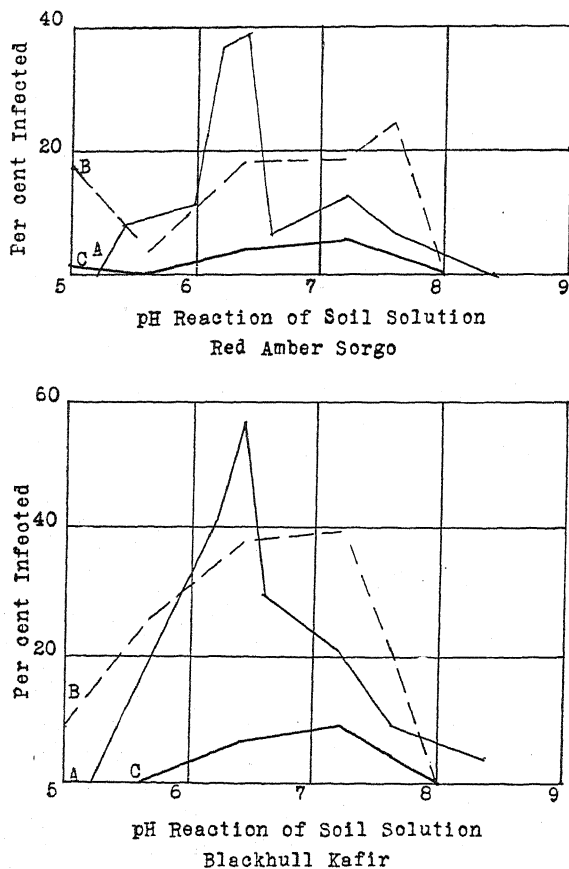
Blackhull Kafir gave the highest percentage of infection (41.9 percent) in a pH of 6.2. Rather high infections were also obtained at pH values of 6.0, 6.6, and 7.2. There was a decrease in the amount of infection at both higher and lower pH values.

Red Amber Sorgo gave the highest percentage of infection (38 percent) at a pH of 6.2. The percentage of infection at pH 6.6 was lower than that at pH 7.2. Aside from this irregularity, there was a decrease of infection in higher and lower H-ion concentrations.

The two experiments in soil gave quite high percentages of infection. Blackhull Kafir had 40 percent infection at pH 6.2 and 56.2 percent infection at pH 6.4. Red Amber Sorgo gave 29.4 percent infection at pH 6.2 and 37.5 percent infection at pH 6.4. It may be noted that the percentage of infection of Blackhull Kafir obtained in sand and soil at pH 6.2 was prac-

tically the same. The percentage of infection of Red Amber Sorgho in soil of pH 6.2 was somewhat less than that obtained in sand of the same pH value.

In 1923, these two varieties of sorghum, Red Amber Sorgho (232) and Blackhull Kafir (223), were again used in experiments on the influence of soil reaction on infection. The methods were similar to those employed in the previous year. The actual pH reactions were 5.0, 5.6, 6.4, 7.2,



TEXT FIG. 7. Influence of pH reaction on infection of Red Amber Sorgho and Blackhull Kafir by *Sphacelotheca sorghi* and *S. cruenta*. A, result with *Sphacelotheca sorghi*, 1922. B, result with *Sphacelotheca sorghi*, 1923. C, result with *Sphacelotheca cruenta*, 1923.

7.6, and 8.0. The sand had a moisture content of 30 percent, and the cultures were grown at the laboratory temperature (19–22° C.). Separate sets of seed were inoculated with *Sphacelotheca sorghi* and *S. cruenta*. The results are given in table 8 and illustrated in text figure 7.



TABLE 8. *Influence of Soil Reaction on Infection of Sorghums by Sphacelotheca sorghi and S. cruenta, 1923*  
(Laboratory temperature, 19°–22° C. Sand moisture, 30 percent)

pH	<i>Sphacelotheca sorghi</i>				<i>Sphacelotheca cruenta</i>			
	Blackhull Kafir (223)		Red Amber Sorgo (232)		Blackhull Kafir (223)		Red Amber Sorgo (232)	
	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.	No. Plants	Percent Inf.
5.0.....	45	8.8	32	18.7	28	0	50	2.0
5.6.....	53	24.5	49	4.0	57	0	50	0
6.4.....	49	36.7	66	18.1	47	6.3	67	4.4
7.2.....	126	39.6	96	17.7	70	8.5	129	6.2
7.6.....	56	21.4	36	22.2	45	4.4	32	3.1
8.0.....	12	0	11	0	6	0	4	0

*Results with Sphacelotheca sorghi.* The highest percentage of infection (39.6 percent) with Blackhull Kafir was obtained at pH 7.2. A very high percentage (36.7 percent) was obtained at pH 6.4, and rather high infections also were obtained at pH 5.6 and 7.6. Only 8.8 percent of the plants at pH 5.0 were infected, and no infection resulted in the culture with pH 8.0.

Red Amber Sorgo gave more irregular results. There were 18.7 percent of the plants infected at pH 5. There was a drop to 4 percent of infected plants at pH 5.6. In the higher concentrations the results were 18.1 percent infection at pH 6.4, 17.7 percent at pH 7.2, and 22.2 percent at pH 7.6.

*Results with Sphacelotheca cruenta.* Both Blackhull Kafir and Red Amber Sorgo gave low percentages of infection with *Sphacelotheca cruenta*. Negative results were obtained with Blackhull Kafir at pH 5.0, 5.6, and 8.0, the highest infection (8.5 percent) being secured at pH 7.2. Red Amber Sorgo gave negative results at pH 5.6 and 8.0. An infection of 2 percent was obtained at pH 5.0. The highest percentage of infection (6.2 percent) occurred at pH 7.2. The number of plants of both varieties at pH 8.0 was small, since poor germination was obtained in this soil condition.

The results for the two seasons indicate that, under the conditions of soil moisture and temperature used, a slightly acid soil reaction is most favorable for infection. Rather high percentages of infection, however, are obtained over a wide range of H-ion concentrations. Blackhull Kafir has responded more uniformly to the soil reaction than has Red Amber Sorgo. The behavior of the two varieties toward *Sphacelotheca cruenta* is similar to their behavior toward *S. sorghi*.

The differences in the pathological effects of the two kernel smuts on the sorghums, as previously described by the senior author, were noted in the varieties grown in these experiments. Plants infected by *Sphacelotheca cruenta* headed out earlier; the stems were shorter with fewer nodes, and tillered and branched more than normal plants or than those infected by

*S. sorghi*. These differences were more evident in Valley Kaoliang than in the dwarfed Darso and Blackhull Kafir.

Our counts in the field were based upon infected plants and in all cases on plants whose terminal heads were infected. It may be noted that no plants infected by *Sphacelotheca sorghi* were observed in the rows where the seed sown had been inoculated with *S. cruenta*, nor were there any cases of plants infected in the typical manner by *S. cruenta* in the rows where *S. sorghi* had been used for inoculating the seed.

The writers, however, made the interesting observation that several plants whose terminal panicles were sound later had lateral panicles infected with *Sphacelotheca cruenta*. These were especially numerous in Valley Kaoliang and in parts of the plot where spores from adjacent plants infected by *S. cruenta* earlier had been disseminated. Such lateral infected panicles also were observed in several of the rows which were originally inoculated with *S. sorghi*. Usually only one infected lateral head was observed, but occasionally there were two or more. Sometimes sound lateral heads were found above or below the infected one. These plants in no way differed in height or other characters from the ordinary normal plants. The writers believe that the occurrence of these lateral infected heads is best explained on the assumption that secondary local infection of panicles by *S. cruenta* may take place. In this smut we may have not only systemic infection by means of spores carried on the seed, but also local infection of panicles by spores early disseminated in the field.

The results of the experiments described above indicate that infection of susceptible varieties of sorghums may occur over a wide range of temperature. In every case infection was secured at 15° C., and in nearly all cases at 35° C. Our results indicate a wider range for infection than those reported by Kulkarni<sup>3</sup> for *Sphacelotheca sorghi*. Further, infection occurred over a wide range of soil moistures, although a much larger percentage of infected plants was obtained in the lower moistures. Soil reaction was also a factor of great importance. While infection occurred over a rather wide range of pH values, slightly acid soils were the most favorable for a high percentage of infection.

The results with the two species of sorghum smuts, *Sphacelotheca sorghi* and *S. cruenta*, were quite similar. They responded in similar fashion to temperature, moisture, and soil reaction in their infection of susceptible varieties.

The resistant varieties of sorghums grown at a number of different temperatures and moistures consistently maintained their resistance to infection.

<sup>3</sup> Kulkarni, G. S. Conditions influencing the distribution of grain smut (*Sphacelotheca sorghi*) of Jowar (Sorghum) in India. Agr. Jour. India 17: 159-162. 1922.

## DOES LIGHT DETERMINE THE DATE OF HEADING OUT IN WINTER WHEAT AND WINTER RYE?

J. ADAMS

(Received for publication December 17, 1923)

Some experiments were carried out in the autumn of 1922 and the spring of 1923 to determine whether the time of flowering of winter wheat and winter rye bore any relation to exposure to light. The experiments were carried out in a greenhouse, the natural period of daylight being supplemented by electric light at night. It was found that the pots of winter wheat subjected to electrical illumination headed out 27 days earlier than those exposed to daylight only, while winter rye headed out 45 days earlier as the result of the extra illumination.

In order to determine whether a similar result could be secured under daylight only, by sowing the seed at different dates, the experiments outlined below were undertaken. With the exception of those of Wanser (6), very few experiments appear to have been made on the subject, although certain investigations made by other workers on wheat and other plants have some bearing on it. Johnston (3) planted seeds of buckwheat in a greenhouse at intervals of a fortnight throughout the entire year, his observations on each set of plants terminating when they were 4 weeks old. He found that the maximum number of flowers was produced by the plants examined between April 24 and May 22, and again between August 28 and September 25. In January and February, and again in July, the number of flowers produced was at a minimum. The greatest dry weight was attained by the plants examined on May 22 and the least dry weight by those examined on January 29, the figures in the latter case being about one twelfth those in the former.

Jardine (1) states that

From field observations, it is clearly evident that Turkey winter wheat stools very heavily when sown early in the season. As the season advances and the weather becomes cooler, less and still less stooling takes place.

In the experiments of Schafer, Gaines, and Barbee (2) at Pullman, Washington, with Hybrid 128 and Red Russian varieties, seeding was done at intervals of 15 days from August 1 to December 1. They found that the crop sown on September 1 gave the highest yield in each case. They further remark that

The true winter wheats when planted in the spring remain as green grass clumps during the entire summer and do not head out until the second year, or at best send up only a few straggling heads in the fall. Such varieties may be planted at any time during

the fall or winter, however, and develop a normal crop the following summer. Hybrid 128 when seeded from October 8 to December 24 showed a certain amount of injury, but it was comparatively hardy. When seeded later than March 11 it failed to head out. The latest date at which a winter variety may be seeded in the spring yet produce mature plants varies with the climatic conditions and variety. Spring varieties should not be seeded in the fall because of the danger of winter killing and winter varieties should not be seeded in the spring because they are likely not to head out.

In Thatcher's experiments (5) at Wooster, Ohio, seeding was done at intervals of one week from August 31 to October 27. He found that the greatest yield was from the crop seeded on September 21-22, and states that "the best date for seeding wheat varies somewhat with the latitude."

Davidson and Stapledon's results (4) with Yeo oats in Wales are similar to those obtained with winter wheat. They found that the same seed sown in spring on ten different farms failed to produce a crop although the seed when tested gave a germination of 97 percent. The failure they attribute partly to climatic conditions and to the fact that the variety was a winter one.

According to Burr and Stewart (7), winter wheat sown on September 22 at Lincoln, Nebraska, headed out on June 1 and gave a yield of 36.5 bushels per acre. Wheat sown at various intervals after this date headed out a little later and gave a gradually decreasing yield. That sown on November 25 headed out on June 14 and gave a yield of 17.0 bushels, while spring wheat headed out on June 12 and gave a yield of 16.2 bushels per acre.

Wanser (6) writes:

In the light of the work of Garner and Allard and of the results secured by the writer, all of this evidence indicates, in the case of winter wheat, the stimulus for jointing to be a critical photoperiod having a maximum limit. The development of winter wheat requires a critical photoperiod for jointing and also a separate and distinct critical photoperiod for heading. Photoperiodism, therefore, is the key to the distinction between winter and spring wheats.

#### EXPERIMENTS WITH WINTER WHEAT

The variety used was Kharkov. Two pots were sown with seed in a greenhouse at Ottawa on March 5, 12, 19, and 26, and on April 3, 9, 16, and 23. The plants were taken out of the pots and planted in open ground on May 15, 1923. For comparison a row was sown in the open ground on April 30. The particulars regarding each of the 9 sets are as follows, the 2 pots in each sowing being labeled *A* and *B*.

1*A*. Seed sown on March 5, 1923. There were 6 plants. One plant came into ear on July 10, while on September 11 there were 51 ears altogether.

1*B*. Seed sown on March 5. There were 6 plants. Several plants were in ear on July 4, while on September 11, 57 ears had been produced.

2*A*. Seed sown on March 12. There were 6 plants. On September 11, 50 ears had been produced.

2B. Seed sown on March 12. There were 5 plants. On July 10 one plant was in ear, while on September 11, 49 ears had been produced.

3A. Seed sown on March 19. There were 4 plants. On September 11, 39 ears had been produced.

3B. Seed sown on March 19. There were 4 plants. On September 11, 16 ears had been produced.

4A. Seed sown on March 26. There were 4 plants. On September 11, 15 ears had been produced.

4B. Seed sown on March 26. There were 6 plants. On September 11, 16 ears had been produced, and on October 27, 2 more ears had been produced.

5A. Seed sown on April 3. There were 6 plants. On September 11, 3 ears had been produced, and on October 27, 6 more ears had developed.

5B. Seed sown on April 3. There were 5 plants. On September 29 none were in ear.

6A. Seed sown on April 9. There were 5 plants. On September 29 none were in ear.

6B. Seed sown on April 9. There were 4 plants. On September 29 none were in ear.

7A. Seed sown on April 16. There were 6 plants. On September 11 one was in ear, while on October 13, 2 ears altogether had been produced.

7B. Seed sown on April 16. There were 6 plants. On September 29 none were in ear.

8A. Seed sown on April 23. There were 5 plants. On October 27 one ear had developed.

8B. Seed sown on April 23. There were 6 plants. On September 29 none were in ear.

9. Seed sown in open ground on April 30. On September 29 none were in ear.

#### EXPERIMENTS WITH WINTER RYE

The variety used was common winter rye, and the particulars of sowing and planting out were exactly the same as in the case of winter wheat.

1A. Seed sown on March 5, 1923. There were 4 plants. Some plants were in ear on July 10, and on September 11, 36 ears had been produced.

1B. Seed sown on March 5. There were 4 plants. On June 11 one plant was in ear, while on September 11, 35 ears had been produced.

2A. Seed sown on March 12. There were 3 plants. On September 11, 13 ears had been produced.

2B. Seed sown on March 12. There were 3 plants. On September 11, 2 ears had been produced.

3A. Seed sown on March 19. There were 3 plants. One plant came into ear on July 4, and on September 11, 21 ears had been produced.

3B. Seed sown on March 19. There were 3 plants. One plant came into ear on June 18, while on September 11, 20 ears had developed.

4A. Seed sown on March 26. There were 2 plants. On June 11 one plant came into ear, and on September 11, 24 ears had been produced.

4B. Seed sown on March 26. There were 3 plants. On September 29 one ear was produced, and on October 13 a second one had developed.

5A. Seed sown on April 3. There were 4 plants. On September 11, 2 ears had been produced, and on September 29, 2 more had appeared.

5B. Seed sown on April 3. Only one plant grew, which produced 2 ears on September 29.

6A. Seed sown on April 9. There were 4 plants. No ears had appeared on September 29.

6B. Seed sown on April 9. There were 3 plants. On September 11, 12 ears had been produced.

7A. Seed sown on April 16. There were 4 plants. On September 29 no ears had been produced.

7B. Seed sown on April 16. There were 4 plants. On September 29 no ears had appeared.

8A. Seed sown on April 23. There were 5 plants. On September 29 one ear had appeared.

8B. Seed sown on April 23. There were 5 plants. On September 29 no ears had appeared.

9. Seed sown in open ground on April 30. On September 29 no ears had appeared.

It appears from the above figures that most of the plants raised from seed sown at intervals in March headed out before the close of the season, the results being more pronounced in the case of winter wheat than in that of winter rye. From the next four sowings made in the greenhouse in April very few ears were obtained, or in some cases none at all. None of the plants raised from seed sown in the open ground on April 30 headed out before the close of the season. In other words, the plants sown first headed out first and the total duration of heading out in the experiments in question extended from June 11 to October 27.

In the experiments carried out during 1922-1923 with electrical illumination, the seeds of the same two varieties of winter wheat and winter rye were sown on October 31, 1922. The pots of winter wheat illuminated headed out in April, while those exposed to natural daylight headed out only in May. In the case of winter rye, the illuminated plants headed out on the last day of February and the first day of March, while the daylight group did not head out until the middle of April.

Depending therefore on the date of sowing, the amount of illumination, and the temperature, winter wheat and winter rye can be induced to head out at any time from March to October.

From the experiments above described and from many others the conclusion is drawn that light and heat are to a certain extent interchangeable in a plant's economy. That is to say, if a plant requires for the completion



of its life cycle a certain number  $L$  of hours of light of a definite intensity  $I$  and a certain number  $H$  of heat units, then the same result may be attained by  $L - l_1$  hours of light and  $H + h_1$  heat units, or by  $L + l_2$  hours of light and  $H - h_2$  heat units, always provided that the intensity of the light and the temperature fall between the minimum and maximum figures for active growth of the plant.

#### SUMMARY

Seeds of winter wheat and winter rye were sown in a greenhouse at Ottawa at intervals of about a week from March 5 to April 23, and the resulting plants were set out in the open ground as soon as the weather became suitable. For comparison, one row of each was also sown in open ground on April 30.

Those sown first headed out the same season, while the plants derived from the last sowings as well as those sown in the open ground did not head out the same season.

The conclusion is drawn that both winter wheat and winter rye require a longer growing season than spring varieties of the same species, and that this growing season is not sufficiently long in the latitude of Ottawa for heading out if sown in the open ground in spring. In this respect their behavior resembles that of the class of winter annuals or biennials.

Both light and heat determine the time of heading out of the two species here discussed, both these climatic factors being of equal importance and, to a certain extent, interchangeable.

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CENTRAL EXPERIMENTAL FARM,  
OTTAWA, CANADA

#### NOTE

The plants referred to above were left undisturbed in the ground over winter. In the case of winter wheat all the March-sown plants were dead, having apparently completed their life history, while the April-sown plants headed out between June 11 and June 16, 1924.

In the case of winter rye, the March-sown plants headed out between May 28 and June 9, 1924, while the April-sown plants headed out between May 28 and June 5, 1924.





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## AGE AND AREA FROM THE VIEWPOINT OF PHYTOGEOGRAPHY<sup>1</sup>

H. A. GLEASON

When Darwin began developing in his mind the general principles of his theory of natural selection, he kept his ideas well to himself and did not offer them to a critical public until they had become well aged and seasoned, so that they were then able to offer a very sturdy resistance to the determined attacks of scores of critics. Dr. Willis, on the other hand, seems to have allowed the age-and-area hypothesis no more than five years to grow and develop its strength before turning it out into the world to suffer the slings and arrows of numerous critics. But it was not left entirely to the sport of its foes; Willis stood, and still stands, always behind it, ready and willing at any time to spring to its defense. A score or more papers in its support show his activity during the past sixteen years. In some of them he appeals to our logic, in some to our philosophy, and in others to our common sense. In many he relies on figures, and marshals columns of numbers and pages of statistics. He deals with square kilometers and archipelagoes; he juggles land bridges and oceanic currents, but in every case he ends, in effect, with such a statement as this: "Here are my figures, gentlemen. They can all be verified. How can you account for these facts except on the basis of Age and Area?"

Far be it from me to object to the use of statistics in matters of plant distribution. I would do it myself, and so have done the numerous critics of Age and Area. They too have brought into action their shock troops of figures, tables, continental floras, Tertiary fossils, and glacial periods, and hurled them against the theory. These criticisms have not been entirely without effect. Each new attack has caused Willis to erect a new defense, which has usually taken the form of an exception to his general statement. These show plainly in his recent book on the subject, in his formal definition of Age and Area. Originally it was, in essence at least, "The area of a species depends on its age;" but now, since one exception after another has been added, it reads:

<sup>1</sup> Read in the symposium on "The Age-and-area Hypothesis" at the meeting of the Systematic Section of the Botanical Society of America at Cincinnati, December 29, 1923. [The *Journal* for October 11: (493-539) was issued October 7, 1924.]

The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, changes of climate from one region to the next, or other ecological boundaries, and the like, also by the action of man, and by other causes.

One can count in this expression of the theorem no less than nine distinct exceptions definitely stated, relating conditions under which the age-and-area principle may not hold. Then, to guard against other criticisms which may arise in the future and which Willis was unable to foresee, he has added the blanket exception "other causes." Lastly, to make his theorem still more nearly impregnable, he has forbidden us to argue against it by citing individual exceptions. In other words, the area of a species depends on its age when it does depend on it, and cases in which it does not must not be mentioned.

Notwithstanding these peculiarities in its statement, Age and Area is an exceedingly important idea. It is one of the few great general statements in phytogeography. It appeals at once to many other botanists besides the phytogeographer. It offers to the systematist a means, whether right or wrong, of determining the rank and relationship of species just at a time when such matters are coming prominently to the fore, and may aid in the miraculous resuscitation of the dry bones of modern taxonomy. To the comparative morphologist, the geneticist, and others interested in the phylogeny of plants, it offers a possible means of determining the ancestry of species, and even of genera and families, and enables them to confirm their conclusions by a new body of evidence. And, more important than these services, it is calling to the attention of all botanists the facts that the fundamental problem of pure botany today is phylogeny, that phylogeny is intimately connected with taxonomy and distribution, and that the problems of phylogeny offer an exceedingly important and comparatively unworked field for botanical research. Willis has expressed this matter beautifully in another paper, where he says:

It is clear that age, area, and size go together, and as age (representing the resultant of the active factors) is the only working factor of the three, whatever phenomena are shown by size should be similar to those shown by space. But size of genera represents evolution, and area represents geographical distribution.

Already Age and Area has had its effect on these two servants of phylogeny. Taxonomy can no longer be the mere description of species from dried herbarium material: it will require the study of species from the standpoint of their origin, as has been done in the recent important publication by Hall and Clements. This idea of course is not new; it has been in the minds of systematists for decades, but Age and Area emphasizes the matter and calls it once more to the attention of some of our species-makers. It can also be said with little exaggeration that, to most botanists, phytogeography

meant nothing more than the determination by observation of the natural boundaries of species, while now, thanks to Willis and his critics equally, phytogeography is a dynamic subject, capable of yielding valuable ideas to the phylogenist and the taxonomist.

Willis' idea of the causes governing the distribution of a species, upon which Age and Area is based, may be condensed into a very few words. A species originates in a small area. It spreads beyond its area of origin. Its present extent depends on the time available for this spread, and, of two related species, the older will have spread the farther. Upon this extraordinarily simple idea the whole theorem of Age and Area is founded.

But is the distributional history of a species as simple as this? Is it possible that every species, or even the average species, has had nothing more remarkable befall it during the whole period of its existence than a mere uninterrupted migration into new areas? Is it not more likely that they have passed through all sorts of vicissitudes? Is it not probable that most species have had times of rapid migration, other times of slow progress; periods when they were retreating from regions formerly occupied, periods when, under the stress of unusual climatic conditions, they were barely able to maintain their existence?

The history of a species is in many ways similar to the history of a nation. A few centuries ago a new people, the Turks, entered eastern Europe. They overran the southeastern part of that continent. They progressed westward as far as Vienna. Then they began to retreat, and for three centuries they have become steadily more and more restricted in area until now they are virtually confined to the vicinity of Constantinople and Asia Minor. Such a history is paralleled in our own country by that of certain tribes of American Indians, originally living near the Atlantic seaboard and now represented only by a few survivors in Oklahoma.

Plants have had a similar history, although the evidence is not always so clear. For some the fossil record is conclusive and for others highly suggestive, but in general the phytogeographer must depend on other sources of evidence in working out the past migrations of plants. In their morphology, in their ecology, in their taxonomic relationships, plants frequently offer excellent evidence on the general location of their origin and the general nature of their movements in the past, and the conclusions drawn from such evidence are frequently quite opposed to those which would be reached through Age and Area alone.

Herein lies one of the chief weaknesses of Age and Area. Migrations, according to Willis, are regularly advancing and serve to extend the range. Retreating migrations, while not explicitly denied by him, are regarded as the exception, since relic endemics, the natural product of such retreats, are considered to be very few in number. But the ecologist knows that every case of succession represents a detailed step in migration, in which one group of species advances and another retreats, and the phytogeographer is able to

trace the accumulated effect of these steps through migrations involving retreats of hundreds of miles. In fact, it might well be argued that, since the Cretaceous at least, retreats and advances of species have been approximately equal in amount.

The whole question of the origin of a species may be passed here, except as it has a space relation. It is of little importance in discussing Age and Area whether species originate by mutation, natural selection, or geographic isolation: at least they originate, and it is not entirely clear to the phytogeographer that they always originate in a small area, as Willis holds. The phytogeographer will insist, however, that the location of the birth of a new species must be within migration distance of the parent. Now, there are many genera well known to all of us, such as *Cercis*, *Liriodendron*, and *Hicoria*, in which the few existing species are separated by considerable distances, and this condition necessarily postulates, at some time in the history of that genus, a retreating migration by one or more of its component species, by which the original continuity of distribution was broken. And how many hundreds of other species may have undergone similar retreats from an older and wider range, without leaving any isolated relatives behind to tell the tale? In a tropical continental area, which has suffered little climatic fluctuation for a long period of time, such retreating migrations may easily be the exception, but in a glaciated region like our own, which has passed through extraordinary changes in its climate in comparatively recent times and which even now seems to be subject to climatic variation, such retreating migrations must be the rule rather than the exception.

During the various advances of the continental ice-sheet into the region of the middle west, the conifers of the Lake Superior region and their accompanying herbaceous species seem to have been compressed into a narrow strip along the glacial boundary. At each retreat of the ice, these plants moved to the northward, expanding as they went into a broad belt, since their advance to the north was more rapid than their retreat from the south. The next glacial advance reversed this process, and species that had become old during an interglacial stage became apparently young once more.

Our deciduous species of the southeast have had a similar but reciprocal history. Where was the osage orange living when the Illinoian ice-sheet had moved southward as far as Cincinnati? Certainly not on the ice-covered land to the north. Yet in the following interglacial stage it migrated as far north as Toronto. Other glacial advances ensued, and the osage orange, driven out of Canada, finally retreated into the Ozark Mountain region, from which it has not yet attempted another sally into the northern states.

Here, therefore, we have examples of retreating migrations affecting whole floras, although it is essential to the general validity of Age and Area that migrations be always forward.

Among the hundred or more species of trees that have moved northward in post-glacial time upon the glaciated region of our northern states, no genus is more widespread than the oaks. All the oaks have about equal ability in migration and undoubtedly belong to an old genus. Many of them live as freely on the unglaciated parts of the country as on the glaciated. Of our common white oaks, the overcup oak has barely crossed the glacial boundary, the post oak has reached southern Iowa, the white oak southeastern Minnesota, and the bur oak has migrated as far as northern North Dakota. They have all had the same opportunity to migrate, but some have done it more successfully than others. Similar differences in migratory ability may be found among the chestnut oaks, the black oaks, and many other entirely different groups, which in themselves satisfy Willis' stated requirements as to number and relationship. And yet the validity of Age and Area depends largely on the stated premise that related species migrate with essentially the same rate of speed.

If we are willing to believe that certain species migrate more slowly than their near relatives, can we not logically extend our belief and say that some do not migrate at all? Among the richest locations for endemism are isolated mountain peaks, from which migration is possible only by a descent into a different environment or by a prodigious jump to the next peak. The same condition is true of island endemics as well. Willis apparently considers all such endemics as young, because of their restricted ranges, but is it not easily conceivable that many of them are old species which have never had a chance to migrate?

The preceding statement refers to cases of physical isolation which are at once apparent. But there are other cases of isolation or of limited migration due to lack of plasticity in the physiological requirements of the species. Willis has built largely on his records of the variation of range in the New Zealand endemics of the same genus, and concludes that those endemics with the wider ranges are the older. He shows, too, that the "wides," those species living also outside of New Zealand, have a wider range in those islands than the endemics. Is it not just as easy to suppose that differences in physiological plasticity have given some species the wider range, no matter what their age? And is it not equally easy to conclude that some of the so-called "wides" of New Zealand, which Willis considers to be immigrants, actually originated in New Zealand and, because of a higher degree of physiological plasticity, have also been able to colonize in Australia?

The speaker has recently published an account of the probable specific evolution of the North American species of *Vernonia*, using both morphological and geographical evidence for his conclusions. Tracing the various species from South America north through the West Indies, and through Central America and Mexico into the United States, he finds a gradual disappearance of the primitive characters of the genus toward the north.



Of any pair of closely related species, based on morphological similarity, the one with the more primitive structures always occupies the more southern range. There is accordingly good ground for concluding that the genus entered North America from the south and that migration has been accompanied by evolution, so that the younger species in general occupy the more northern part of the generic range. Yet in many cases the younger species, without primitive characters, have far wider ranges than their most closely related older species with primitive structures. In fact, the three most advanced and ostensibly youngest species of all, *V. noveboracensis*, *V. altissima*, and *V. missurica*, have ranges vastly exceeding the more southern and older species from which they were apparently derived. Since their general habit and migratory ability are essentially uniform, one can conclude only that, in this case at least, morphological and geographical evidence affords a better means of determining relative age of species than their area alone.

If species migrate with the same speed and meet with the same barriers or none, and if their migration is always one of advance, never of retreat, then the age-and-area theorem holds. Certainly there must be some genera which have had such a history, and I have no doubt there are more such species proportionately in the tropics and the subtropics, where Willis has secured most of his evidence, than in our glaciated country. But if related species migrate at different rates, if they migrate backward as well as forward, if some never migrate at all, if some can ultimately cover a great area because of great physiological plasticity while others never get beyond restricted limits, and the phytogeographer thoroughly believes that such conditions exist for thousands of species and genera, then for such plants Age and Area can never be valid. In other words, Age and Area succeeds when it succeeds, fails when it fails, and the conclusion from certain cases that it is generally applicable is logically a *non sequitur*.

NEW YORK BOTANICAL GARDEN



## AGE AND AREA AS VIEWED BY THE PALEONTOLOGIST<sup>1</sup>

EDWARD W. BERRY

May I preface what I have to say on the special phase of the question to which my paper is devoted by a few more general observations?

That the book, as well as the special papers, on Age and Area are stimulating is shown by the interest and discussion which they have aroused in botanical circles, and I am sure that the hypothesis will have served a useful purpose if it causes us to re-examine the basis for some of our beliefs.

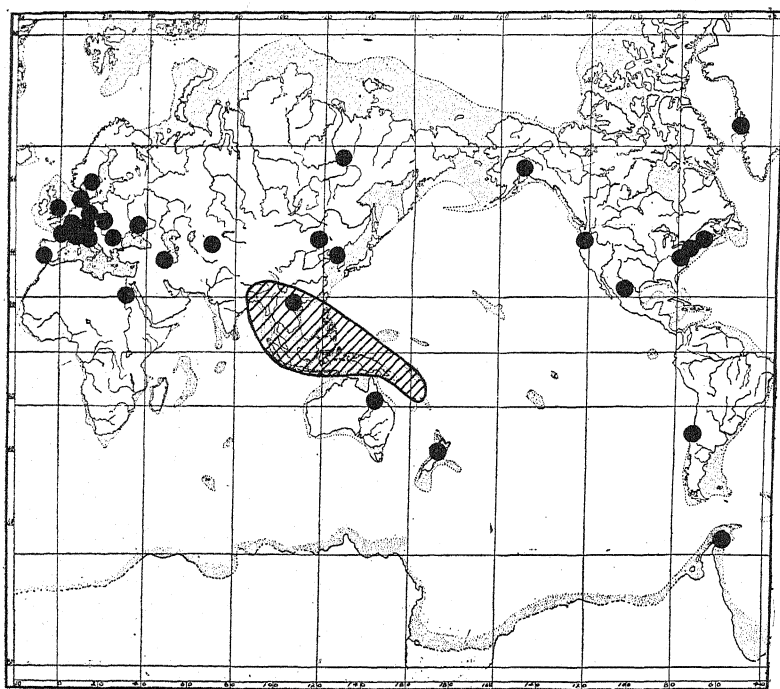
Willis' ideas, it seems to me, are embalmed in a very complicated style, and he makes a great many assertions which are not and can not be substantiated. He claims that the distribution of any species of plant or animal is due solely to the length of time that the species has been in existence, and is not at all dependent on any advantages which it may possess in such matters as production, viability, or dispersal of seeds. Success in life is not dependent on the character of the roots, the nature of the conducting or storage tissues, the form and character of the leaves, the nature of the flowers, or on any other morphological or physiological structures or functions, but is solely a matter of age. In other words, Willis does not believe in those processes which may be grouped under the term *adaptation*, and he, of course, has no use for natural selection.

According to his hypothesis large genera are older than small ones, and he claims that relic endemics constitute but one percent of the existing flora. This figure seems to me too small, but I have not sought to test it, since I have an inborn feeling that mathematics, especially in the field of statistics, can prove anything, and that the value of such proof rests after all on the judgment of the mind behind the figures. The fact that Professor James Small, on analysis by age-and-area methods, finds that the great alliance of the Compositae originated in the mountains of northwestern South America at a time when there were neither mountains nor even land in that region, but seas, does not add to my confidence in the general method. It seems to me to be obvious that, in the abstract, the older the species the more extended should be its range. Reflection will show, however, that the problem is not so simple, and that hosts of other factors are involved in distribution, and that most of these latter are more potent factors than is the time element.

Willis explains extinction by the phrase "exhaustion of vitality," which to me does not mean anything. Extinction is really the rock on

<sup>1</sup> Read in the symposium on "The Age-and-area Hypothesis" at the meeting of the Systematic Section of the Botanical Society of America at Cincinnati, December 29, 1923.

which the whole hypothesis is wrecked. May I remind you that I have conducted graduate work in historical geology and paleozoology for over a decade, so that I may be presumed to have some slight acquaintance with the animal as well as the plant phase of the history of organisms, and I have no hesitation in stating most categorically that throughout the entire known history of organisms we observe radiation accompanied by adaptations leading to specialization, and that a constant flux of environments—both physical and organic—results invariably in the extinction of the more specialized and hence less plastic forms. Many groups—families, orders,

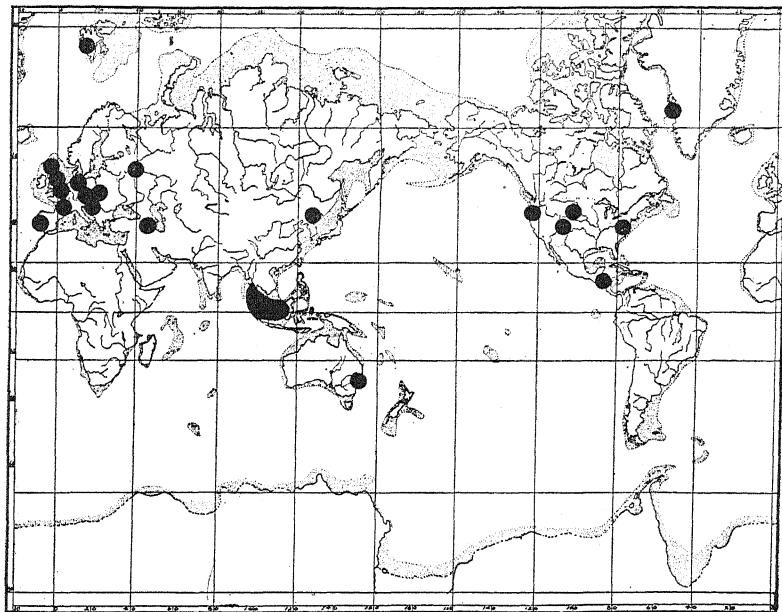


TEXT FIG. 1. The present distribution (barred area) and the location of fossil remains (solid circles) of Dipteriaceae.

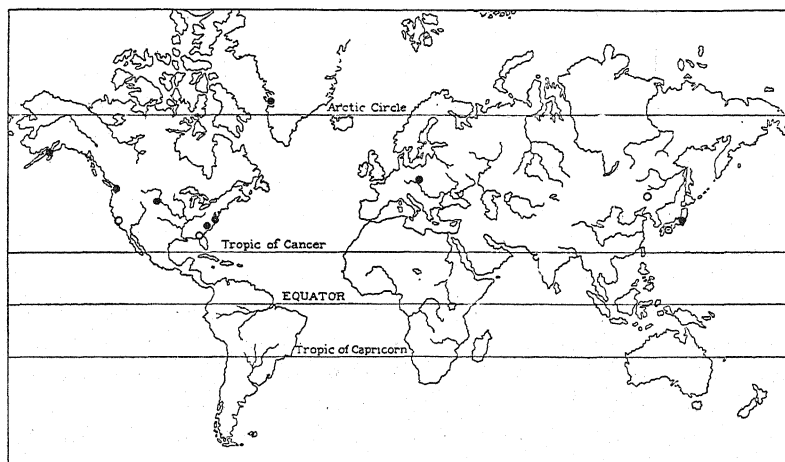
and whole phyla—lived for longer periods than have the existing higher animal and plant stocks, but are today entirely extinct. I will not burden you with evidence drawn from the animal side, but if you will examine any work on either vertebrate or invertebrate paleontology, you will find the data upon which this statement rests.

That large existing genera are not necessarily older than small ones may be shown by enumerating some at present exceedingly small groups which are very much more ancient than most of their existing relatives. Among ferns I will mention only *Dipteris* and *Matonia*; among gymnosperms,

Ginkgo, Sequoia, Taxodium, Glyptostrobus, Torreya, Araucaria, and Widdringtonia; among monocotyledons, Nipa; among dicotyledons,



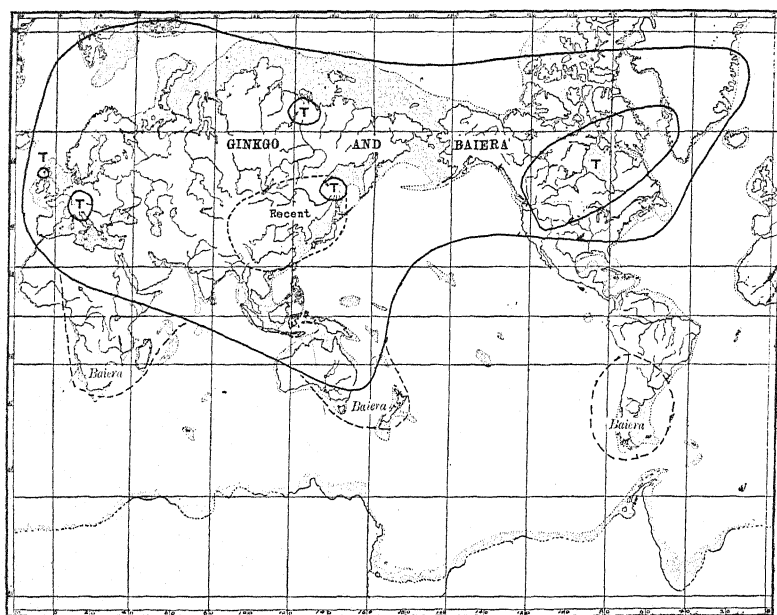
TEXT FIG. 2. The present distribution (irregular black area) and the location of fossil remains (solid circles) of Matoniaceae.



TEXT FIG. 3. The present distribution (hollow circles) and the location of fossil remains (solid circles) of Torreya.

Liriodendron, Sassafras, Juglans, and Cercis. I might enumerate very many more.

The accompanying maps showing the present and past distribution of several types corroborate one another in showing that, outside of certain relatively modern groups, and more particularly the herbaceous forms of, for example, the Leguminosae, Cruciferae, Labiatae, Boraginaceae, Orchidaceae, Compositae, etc., the small, restricted monotypic or ditypic genera in the existing flora have had in every case where something of their history is known a much greater area of distribution in the past than they enjoy at the present time.

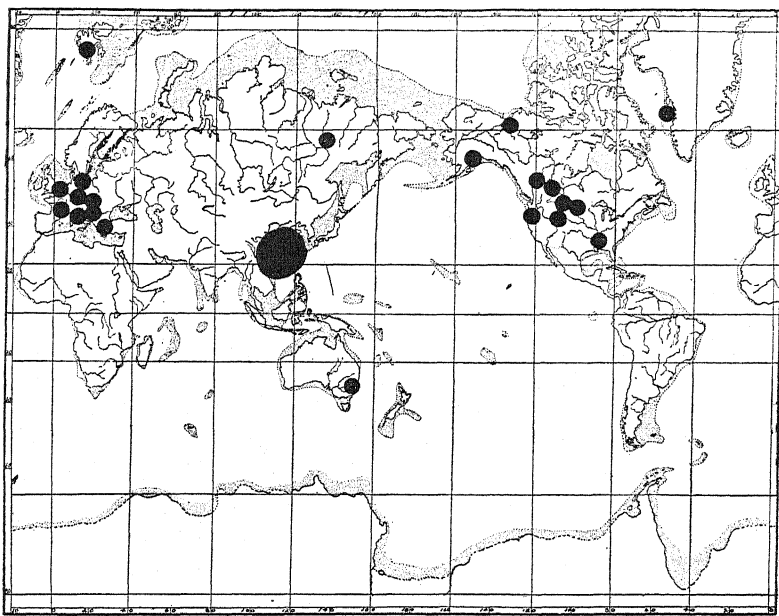


TEXT FIG. 4. Known range of Ginkgo and Baiera in the past. Inner circles (T), Tertiary survivals of Ginkgo. Broken circles, areas in which the existing species were indigenous during the Tertiary.

I realize perfectly that most of these illustrations are expressly excluded by Willis in his insisting on using ten related species in his calculations; and also that it might be claimed that, if past distribution were added to present distribution, these illustrations would fulfill the claims of the hypothesis. At the same time it seems to me that they are not so readily set aside and that they throw considerable doubt on the results set forth in his book. With the exception of Guppy it does not impress me that his collaborators have added much of value in the chapters which they have contributed. This remark is particularly pertinent with respect to the chapter contributed by Mrs. Reid, who speaks for but one class of fossil plant remains, and who has a very imperfect acquaintance with the Tertiary plant history of Europe.

Among the existing ferns, two of the most interesting families are the Dipteriaceae and Matoniaceae, both of which have been discussed from the distributional standpoint by various students. Text figure 1 shows the area within which the four existing species of *Dipteris* are found, and the solid circles show that the family was cosmopolitan in the past, particularly during the Mesozoic. Text figure 2 shows the same for *Matonia*, the two existing Malayan species occupying a very small area, and the fossil occurrences of the family covering all the continents except Africa and South America.

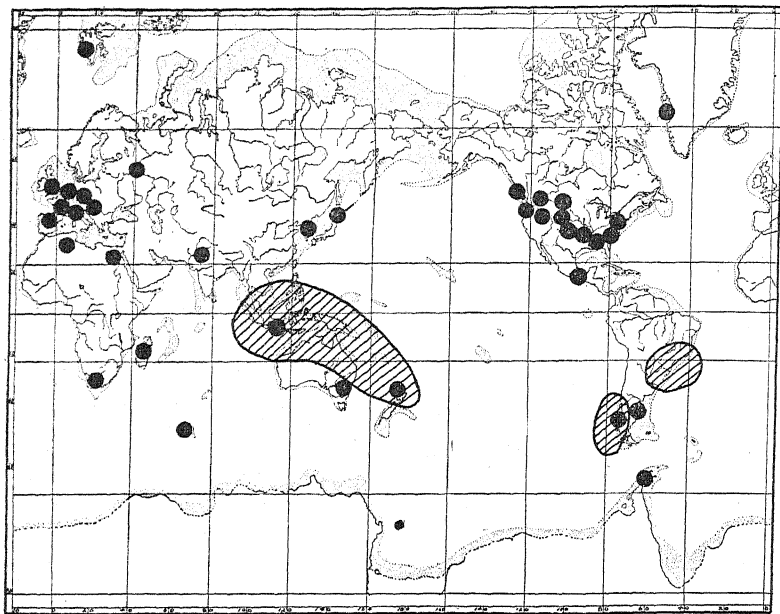
Text figure 3 shows the gymnospermous genus *Torreya* (Tumion), with a restricted modern range (rings) in eastern Asia, Florida, and Cali-



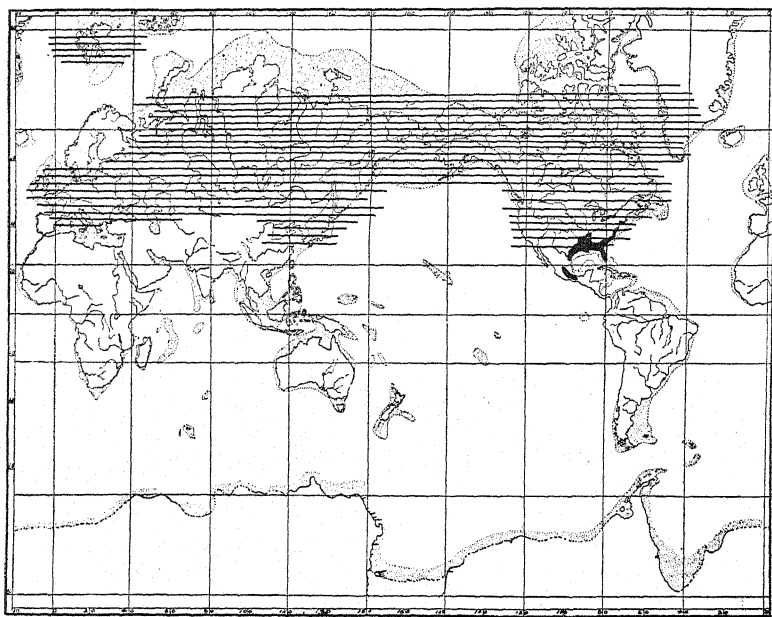
TEXT FIG. 5. The present range (large circle) and location of fossil remains (smaller circles) of *Glyptostrobus*.

fornia, and fossil species (solid circles), mostly Mesozoic, in Asia, Europe, Greenland, and pretty well distributed over North America. In text figure 4 is shown the history of *Ginkgo* and its extinct ally, *Baiera*. The single existing species, often called "a living fossil," is not certainly known outside of cultivation. Its history goes back to Devonian times, and its fossil records are known abundantly from all the continents except South America. As late as the Tertiary it is found widely distributed in Europe, Asia, and North America.

In text figure 5 the existing range of the two modern species of *Glyptostrobus* is shown, much exaggerated as to area, in southern China, and the



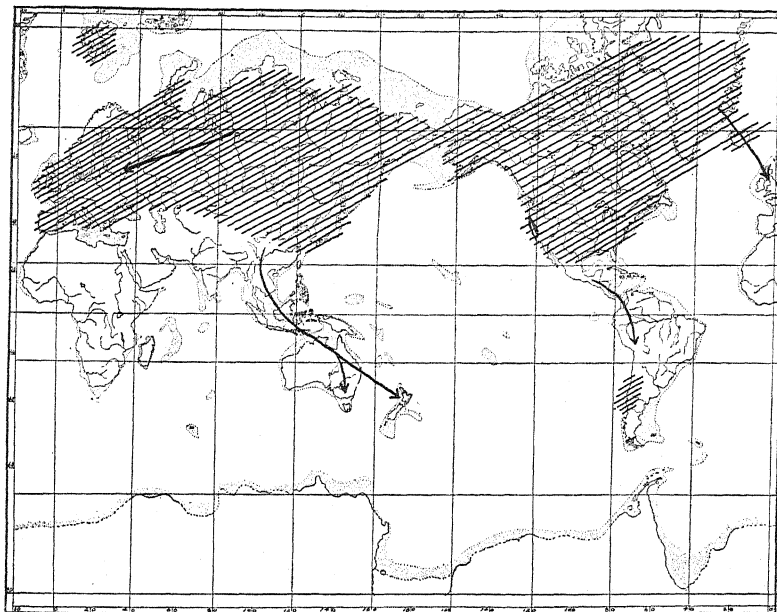
TEXT FIG. 6. The present distribution (barred areas) and the location of fossil remains (solid circles) of *Araucaria*.



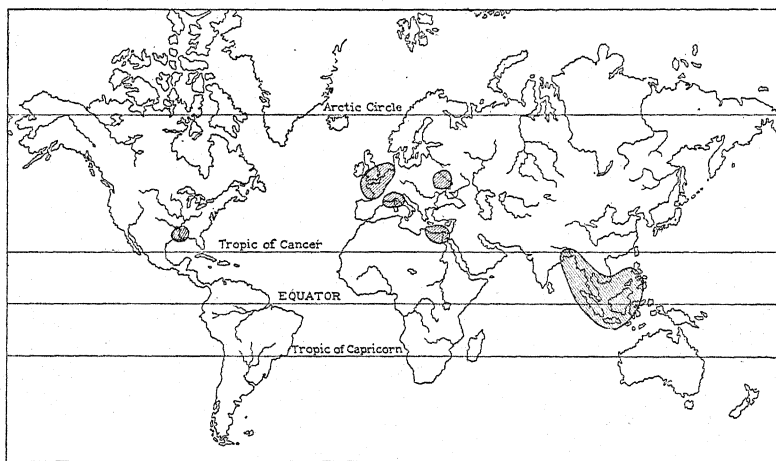
TEXT FIG. 7. The present range (solid black) and past distribution (barred areas) of *Taxodium*.



solid circles show it to have been an exceedingly abundant and practically cosmopolitan type during the Tertiary. Text figure 6 shows a still more



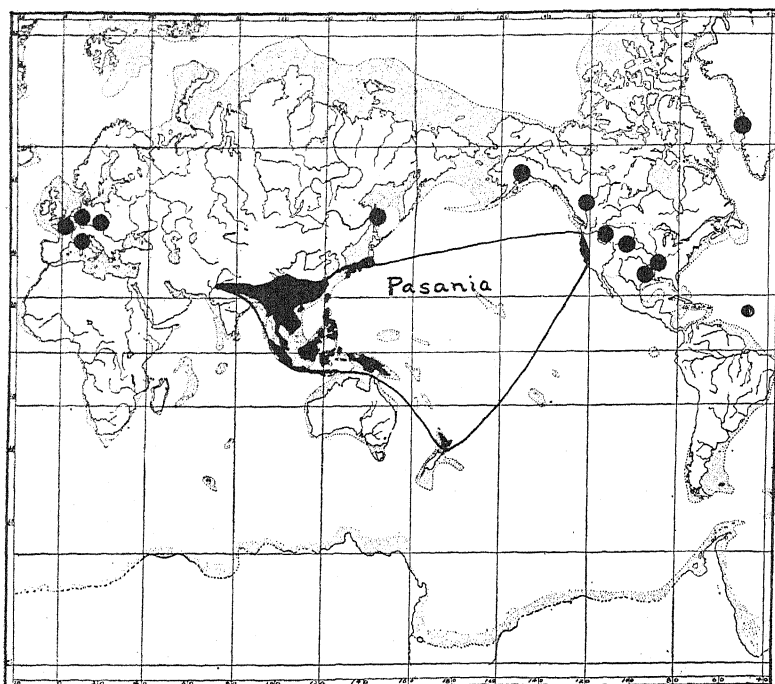
TEXT FIG. 8. The present range (solid black) and past distribution (barred areas) of *Sequoia*, and the probable paths of migration.



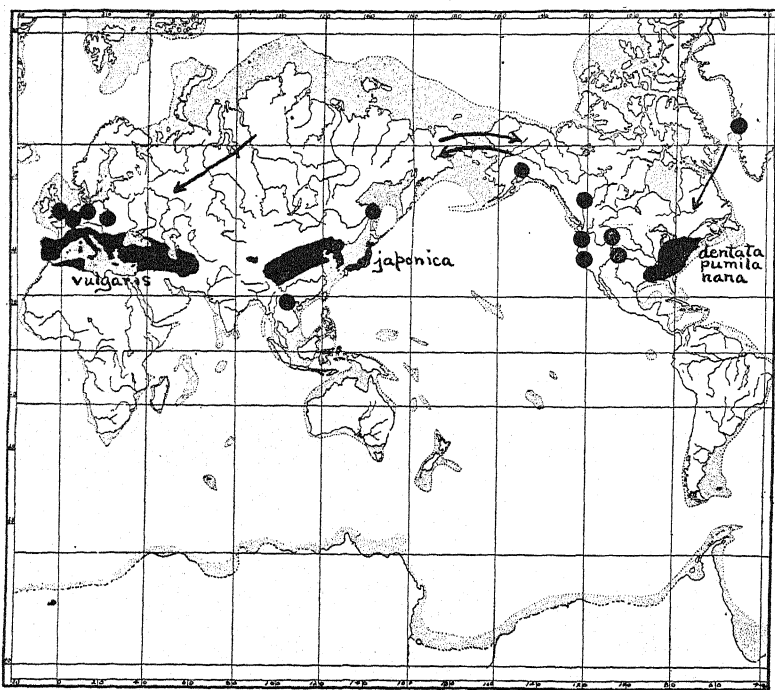
TEXT FIG. 9. The distribution of the single existing species of *Nipa* (Indo-Malayan), and the Tertiary occurrence of *Nipodites* (in North America, Europe, and Africa).

striking contrast between present and past distributions in the case of the very much older genus *Araucaria*.

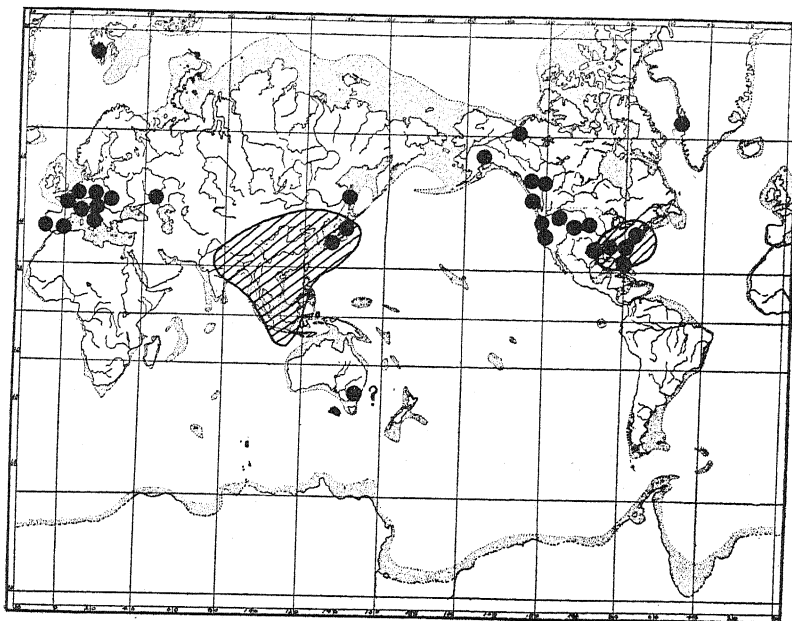




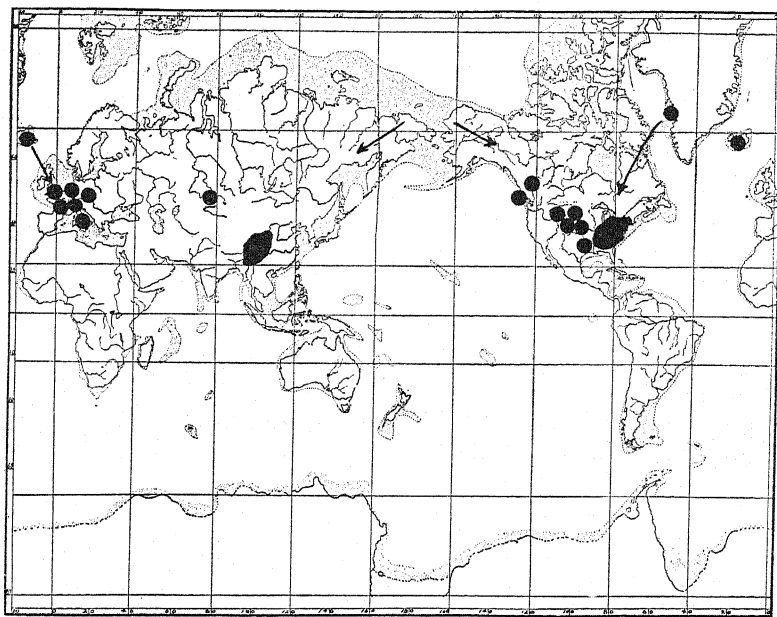
TEXT FIG. 10. The present distribution of *Pasania*, and (in the solid circles) the location of fossil remains of the extinct genus *Dryophyllum*.



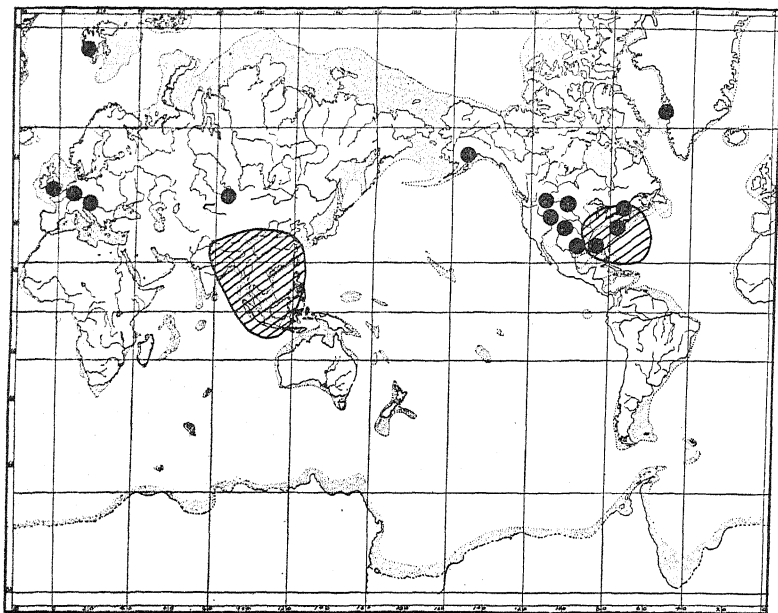
TEXT FIG. 11. The present range (irregular areas) and the location of fossil remains (circles) of *Castanea*.



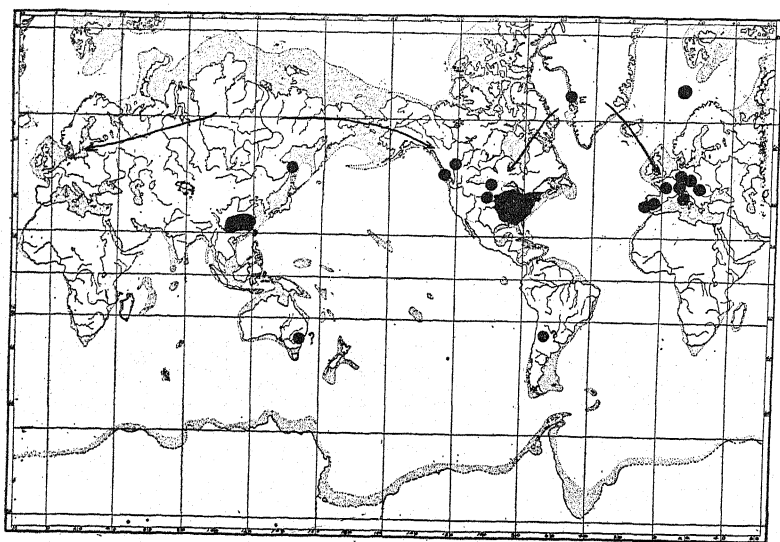
TEXT FIG. 12. The present distribution (barred areas) and the location of fossil remains (solid circles) of *Magnolia*.



TEXT FIG. 13. The present range (irregular areas) and the location of fossil remains (circles) of *Liriodendron*.

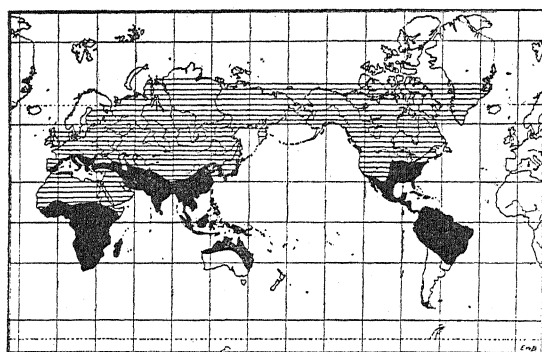


TEXT FIG. 14. The present range (barred areas) and the fossil occurrence (solid circles) of *Nyssa*.



TEXT FIG. 15. The present range (irregular areas) and the fossil occurrence (circles) of *Sassafras*.

Text figure 7 shows a perhaps more striking contrast between the present and the Tertiary distribution of *Taxodium*, and text figure 8, which represents the older genus *Sequoia*, is perhaps still more impressive. Text figure 9 shows the Indo-Malayan range of the present-day Nipa palm, and the oppositely lined areas in North America, Europe, and Africa show its Tertiary occurrences. Text figure 10 shows the range of the genus *Pasania* and the solid circles show the geologic occurrences of the genus *Dryophyllum*, much like, and probably ancestral to, *Pasania* as well as the other *Fagaceae*. In text figure 11 are shown the range of the existing chestnuts and the fossil occurrences of *Castanea*.



TEXT FIG. 16. The present range (solid black) and the fossil occurrence (barred areas) of *Diospyros*.

Text figure 12 gives the same facts for the genus *Magnolia*, and text figure 13 shows how restricted the present range of *Liriodendron* is as compared with its geologic range. Text figure 14 shows the same facts for the genus *Nyssa*, the fossil records in this case being for the most part fruits rather than foliage. Text figure 15 portrays the present range and past range of the small genus *Sassafras*, and text figure 16 illustrates similar facts regarding the large genus *Diospyros*.

I am indebted to the Williams and Wilkins Company for permission to reproduce figures 7, 8, 10, 11, 12, 13, 14, 15, and 16 from my book on *Tree Ancestors*, in which will be found many additional distributional maps that illustrate the same contrasts between present-day restricted range and past extended range.

THE JOHNS HOPKINS UNIVERSITY

## ISOLATION AND ENDEMISM IN NORTHEASTERN AMERICA AND THEIR RELATION TO THE AGE-AND- AREA HYPOTHESIS<sup>1</sup>

M. L. FERNALD

From a purely academic standpoint, it seems perfectly obvious that, given a static world, the longer a plant or a group of plants has existed the more time it will have had to spread and consequently the more space it can have covered. No one can doubt this truism; and if this were all there were to Age and Area the proposition would need no discussion. But, unfortunately, Dr. Willis insists that Age and Area is the general rule in a world which has been far from static, and he has saddled upon his axiomatic proposition a great body of corollaries and from it has deduced a vast number of conclusions, many of which are diametrically opposed to the experiences of other botanists.

In studying Willis' numerous interlocking papers I find myself constantly impressed by the ease with which the great facts of Cretaceous and Tertiary and modern geological history are brushed aside, and by the way in which edaphic factors are swept into the discard. In the time at my disposal only a limited number of Willis' deductions can be discussed, but I have aimed to select a few matters about which I can speak through personal familiarity with the situation.

At the eastern margin of the heavily glaciated region of North America lies an area of much interrupted lands which to a great extent passed unscathed through the Pleistocene glaciation. This is the area immediately surrounding the Gulf of St. Lawrence: the Gaspé Peninsula of Quebec, the Magdalen Islands, Prince Edward Island, northern Cape Breton Island, and western Newfoundland. During the past two decades much of my field work has been devoted to some of these areas, and I wish particularly to draw your attention to two of the regions, the Gaspé Peninsula and the island of Newfoundland. Both have long mountain ranges which stand high above the area of general glaciation, the Shickshock Mountains of Gaspé rising 2,000 to 2,500 feet above the region of general denudation, and the Long Range, which forms the backbone of western Newfoundland, likewise showing signs of general glacial activity only on its lower slopes.

The two regions are, then, nearly unique in the latitudes of extreme Pleistocene glaciation, in that they both have ancient floras which locally outlived the glacial period; but, like the other regions in the glaciated

<sup>1</sup> Read in the symposium on "The Age-and-area Hypothesis" at the meeting of the Systematic Section of the Botanical Society of America at Cincinnati, December 29, 1923.

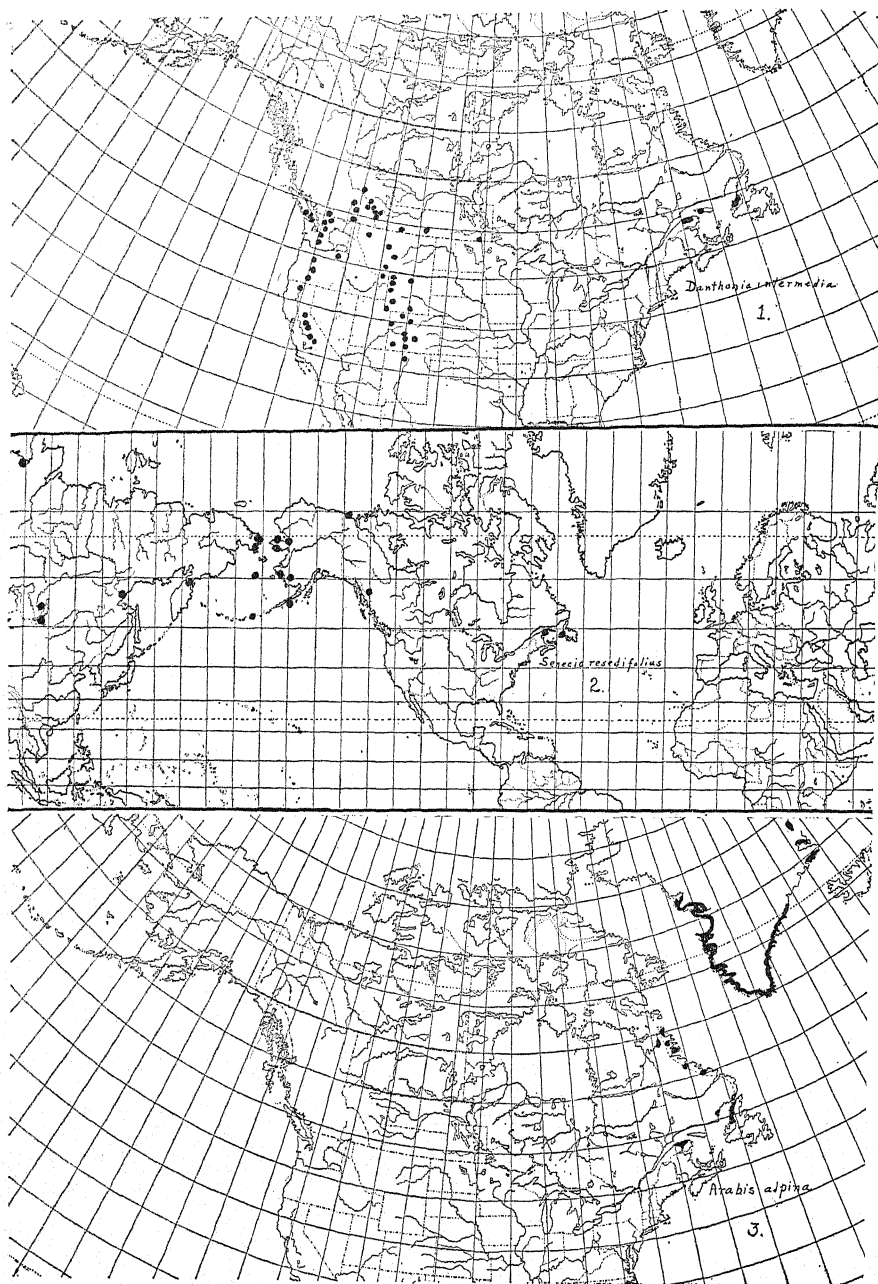
latitudes, upon the general receding northward of the ice-front their lower levels, which had been scoured by local ice-sheets, were invaded by the floras which were then marching north in the wake of the ice. In other words, both regions have an ancient flora and a much younger flora, and by the hypothesis of Age and Area the ancient plants of the two regions should be the widespread ones both within and without the areas, while the youngest to arrive should be of more limited range both within and without these regions.

In studying the flora of Gaspé I have felt justified in treating as the ancient species those which today occur upon the unglaciated mountains above the 2000-foot level (the upper limit of general glaciation) and which are not in the arctic and subarctic regions to the north. If they occur at all generally on Labrador or in the arctic region of eastern America they are treated as belonging to the modern flora of Gaspé, being species which had come south in the Pleistocene and which have now returned largely to northern latitudes, leaving outliers in the south. The plants which extend from New Brunswick or Maine into Gaspé but which are not found in the unglaciated mountain area I have likewise considered as belonging to the modern flora of the Peninsula.

Similarly with Newfoundland; the plants which center upon the Long Range but are unknown northward beyond the closely adjacent corner of Labrador or southwestward in Nova Scotia, New England, Long Island, or New Jersey, I have looked upon as the ancient flora, while all others are younger in their occupation of Newfoundland. But while the plants which have invaded Gaspé in post-glacial times have been forced to make the entrance from the southwest, Newfoundland has had two modern sources for its flora. At the close of the Pleistocene the continental shelf, which now forms the banks off the coast of eastern America, was high in the air and made a nearly continuous sandy coastal plain outside the ice-sheet all the way from our southern states to southeastern Newfoundland. Along this silicious belt, therefore, came the plants of the South, which, upon the retreat of the Newfoundland ice-cap from the southern part of the island, made a start upon the newly exposed land. This southern element is a large one and it includes members of some decidedly austral groups: *Xyris*, *Eriocaulon*, *Gaylussacia*, *Bartonia*, and *Schizaea*.

The arctic plants which reached the high mountains of northern New England seem to have reached Newfoundland by crossing from southeastern Labrador after the local ice had completely left the low region of northern Newfoundland. This is indicated by the fact that the continental ice-sheet, coming off the Labrador Peninsula but not touching Newfoundland, crossed the St. Lawrence to the west of the Gaspé Peninsula, while, as already noted, the region bordering the Gulf of St. Lawrence was only slightly and locally affected by the ice. The arctic plants, forced south in front of the ice, consequently found their way, upon the receding of the ice-front, to the high summits of northern New England and later to those of





MAP 1. Range of *Danthonia intermedia* Vasey.

MAP 2. Range of *Senecio resedifolius* Less.

MAP 3. American range of *Arabis alpina* L.



Gaspé, but not until the low northern end of Newfoundland was clear of ice could they make an effective entrance upon that island. The boreal flora, containing the arctic types which are found on the Labrador Peninsula, was, therefore, the latest to reach Newfoundland.

An illustration or two will make my differentiation clear. The mountains above the generally glaciated areas, in both Gaspé and Newfoundland, are inhabited by *Danthonia intermedia* Vasey (map 1) and *Senecio resedifolius* Less. (map 2), plants which are there perfectly characteristic but are quite unknown on the Labrador Peninsula to the north, though with widespread areas in western North America or Siberia. Such plants, centering on the unglaciated areas of Gaspé and Newfoundland and isolated by a distance of 1500 to 3000 miles from the great mass of the species, obviously can not have migrated into their insular and peninsular eastern areas in post-glacial times from the adjacent continental regions where they do not occur; and, since they center absolutely upon the areas which are unglaciated, they are looked upon as members of the ancient flora of the Shickshocks and the Long Range. *Arabis alpina* L. (map 3), on the other hand, being an arctic type which today is found generally to the north of Gaspé and Newfoundland, is looked upon as a species which could have migrated into those regions in post-glacial time from the surrounding territory, while such a plant as *Schizaea pusilla* Pursh (map 4), of the New Jersey pine-barrens, but more general on the bogs and barrens of Nova Scotia and Newfoundland, is classed as a species which, since the receding of the ice from those areas, must have entered from the south.

With this preliminary explanation, let us examine the ranges inside and outside the Gaspé Peninsula of the indigenous flora of the area. The ranges in the peninsula (and likewise in Newfoundland) are based upon the detailed daily records during many seasons of all plants seen (as well as collected) in every township, river system, lake, or mountain area by myself and the botanists accompanying me, *i.e.*, the records are unusually complete, having been made for the very purpose of indicating exact ranges.

TABLE I. *Flora of the Gaspé Peninsula*

	Ancient Flora of Unglaciated Area		Younger Flora (Post-Glacial)	
	Length of "Wide" Area	Portion of Peninsula Covered	Length of "Wide" Area	Portion of Peninsula Covered
Filices and Lycopods (49 spp.) . . . . .	6,434 miles	.48	11,614 miles	.90
Gramineae (68) . . . . .	4,378	.54	9,063	.83
Carex (92) . . . . .	4,929	.50	8,430	.77
Salix (33) . . . . .	920	.29	3,028	.56
Cruciferae (23) . . . . .	4,025	.44	11,645	.55
	5)20,486	5)2.25	5)43,780	5)3.61
Average . . . . .	4,097	.45	8,756	.72

I have tabulated the ranges in the Gaspé Peninsula (table 1) (approximately 150 miles long) and outside of representative large groups: the ferns and lycopods (49 species), the grasses (68 species), *Carex* (92 species), *Salix* (33 species), and Cruciferae (23 species); and, since the figures for these groups are so uniform, it has seemed useless to continue on through the half dozen other equally large groups. The summaries are presented in table 1.

The plants of the ancient flora of Gaspé cover areas in the peninsula ranging from less than 1 mile (single stations) to 150 miles (the length of the peninsula), with an average length of area in the peninsula of 72 miles, *i.e.*, 0.48 of the region. As a matter of fact, however, the figures, worked out on the principle followed by Willis of measuring the *length* of the range, are by no means accurate, since the *breadth* of the ranges has not been figured, and many species, although following a mountain range or a special type of rock for 40 or 50 miles, have a *breadth* of area which is exceedingly limited. In Newfoundland the computation of area follows a slightly different principle. Newfoundland is not an elongate neck of land like Gaspé, but is a rough triangle with a length in either direction, north-south, or east-west, of approximately 350 miles. Since the southern species entering Newfoundland off the continental shelf to the south might have made their entry almost anywhere along the southern side of the island, and since the northern species coming off Labrador entered at the slender northern tip of the triangle and have radiated east and west as well as south, I have measured both the east-west and north-south diameters of ranges and have averaged the totals of both. Thus, the measurements for Newfoundland are more nearly exact than those for Gaspé.

The five large groups (with 239 species) give in Newfoundland the following results (table 2):

TABLE 2. *Flora of Newfoundland*

	Ancient Flora of Unglaciaded Region		Younger Flora (Derived from Coastal Plain)		Youngest Flora (Derived from Labrador)	
	Length of "Wide" Area	Portion of Island Covered	Length of "Wide" Area	Portion of Island Covered	Length of "Wide" Area	Portion of Island Covered
Filices and Lycopods (44 spp.).....	9,340 mi.	.24	10,467 mi.	.76	15,737 mi.	.86
Gramineae (57).....	6,247	.23	4,796	.58	11,155	.65
<i>Carex</i> (82).....	4,340	.19	5,371	.74	14,286	.84
<i>Salix</i> (26).....	993	.09	2,642	.69	4,107	.42
Cruciferae (30).....	3,871	.24	8,850	.65	10,500	.40
Average.....	<u>524,791</u> 4,958	<u>5.99</u> .20	<u>532,126</u> 6,425	<u>53.42</u> .68	<u>555,785</u> 11,157	<u>53.17</u> .63

Now, these figures might seem very reassuring to supporters of Age and Area whose formula says "the wider the range [of species] outside the wider inside, the narrower the range outside the narrower inside" ("Age and Area," 61); for, of the Gaspé flora, the species which range widest over the world (average, 8756 miles) also have the widest range in Gaspé (0.72 of the area), while the species which have an average wide range of only 4,097 miles likewise have a more limited range in Gaspé (0.45 of the area.) Similarly, the element of the Newfoundland flora which has a wide range of 11,157 miles has likewise a wide range in Newfoundland (0.63 of the area), while the element which has a wide range of only 4,958 miles has in Newfoundland a narrower range (0.20 of the island). But a moment's thought will show that the actual facts are squarely opposed to Age and Area; for in both Gaspé and Newfoundland the most ancient flora of each region has the most limited range and also the most limited "wide" range, while the youngest flora to arrive in each area has two or three times the range both inside and outside these regions.

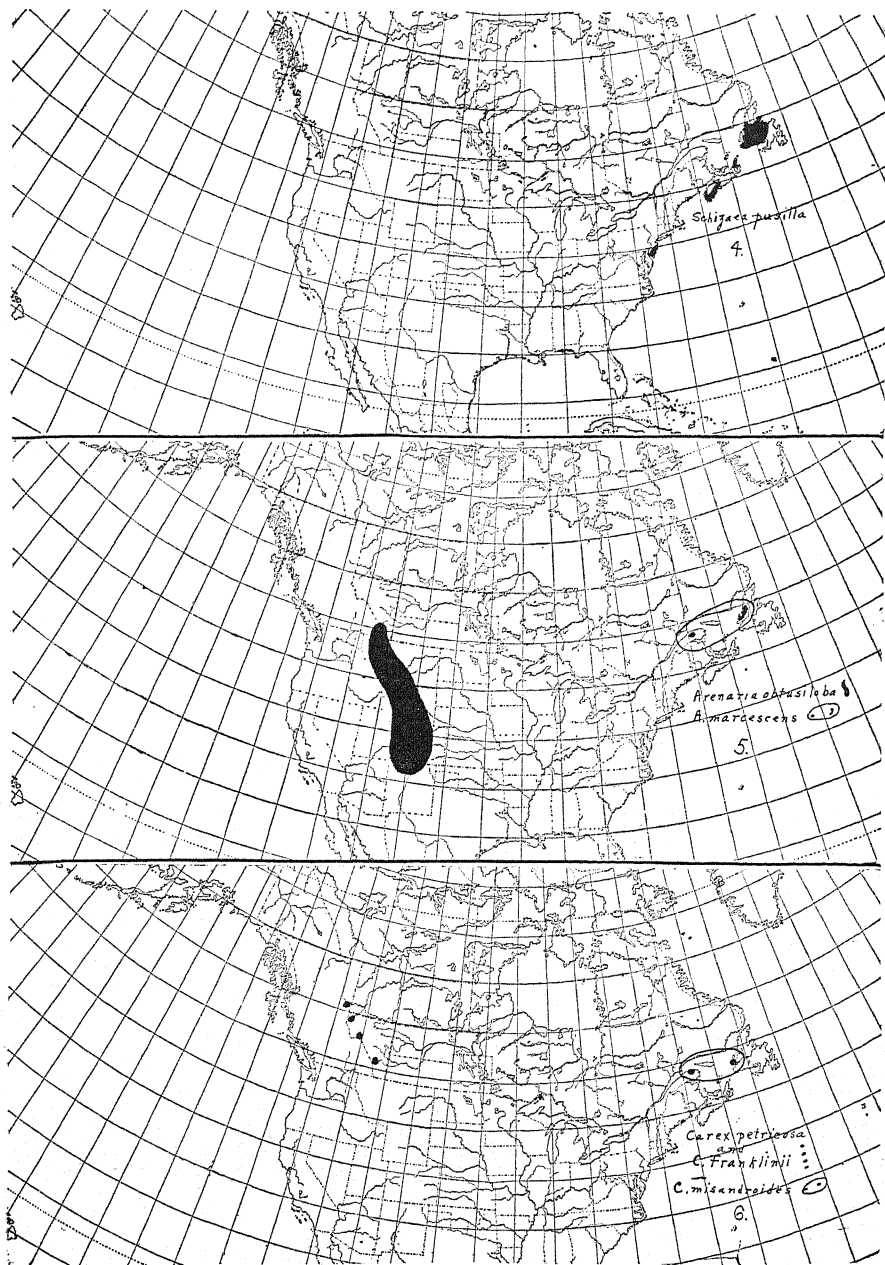
In case of such figures as these, where the species which have the widest range outside a given region have also the widest range within the region and those with the narrowest range outside have the narrowest range inside, Willis says that, without relying upon Age and Area, "to explain such cases the most improbable supplementary hypotheses have to be advocated" ("Age and Area," 84). In the case of Newfoundland the fact is simply this: the very restricted distribution on the island of the species confined to the region centering on the Long Range is associated not only with the unglaciated condition of those tablelands but with the fact that to a considerable extent they are composed of calcareous or magnesian rock and that the plants which center there are such as usually occur on these rocks. Other areas of both calcareous and magnesian rocks occur in the heavily glaciated sections of Newfoundland, but, so far as our information shows, the old plants of the Long Range have apparently failed to take possession of them. The youngest flora on the island consists of the very young group of species which originated in polar regions and, spreading in Pleistocene time, has encircled the northern half of the northern hemisphere. Entering Newfoundland from Labrador, they have found congenial conditions widespread and consequently they are now widespread on the island. This does not seem to be an "improbable hypothesis," but it is surely at variance with the hypothesis of Age and Area.

If we failed to take into account the geological history of these regions and their ecological conditions, the figures, interpreted merely by the rule of Age and Area, would lead diametrically away from the truth. In view of this fact we are justified in wondering if some of the statistics which have been urged in support of Age and Area may not prove, when closely scrutinized by those who take fully into account the historic and ecological factors, quite as deceptive as would be the unexplained figures for Gaspé and Newfoundland.

As a direct deduction from Age and Area, Willis makes the many-times-repeated assertion that endemics are the youngest species and that they are restricted in range simply because they are "too young to have had time to spread abroad" ("Age and Area," 61). Naturally there are several kinds of endemic species; for example, the species with fundamental or deep-seated and essentially unvarying characters of the reproductive organs, commonly isolated from their nearest allies and causing no embarrassment to the systematist; or the plant isolated by considerable distance or by well marked barriers from its congeners and varying from them only in comparatively slight differences in the vegetative characters (size, cutting of leaf, shape of inflorescence, development of pubescence, etc.) but retaining the fundamental characters of the geographically isolated relatives. These are the geographic varieties or subspecies, usually tolerably constant so long as isolation is maintained but not so far segregated as to constitute true species. And again, there is the maze of segregates being set off, often in close proximity to others, in many of our fluctuating and hopelessly inconstant groups: such familiar examples as the European hawkweeds, the European and Eastern American brambles or blackberries, the multitude of segregates of *Crataegus*, the hopeless tangle of variations in the subgenus *Euaster* or in *Artemisia*. In the latter cases most of the species are obviously very young, and too often they are merely the more pronounced nodes in a chain of intergradient variations. Naturally, if these were the only endemics referred to by Willis as so young that they have not had time to spread, there would be no opposition to his interpretation; but he makes it perfectly clear that in his deduction he includes the "Linnaean species," i.e., the full-fledged species with fixed and deep-seated difference in flower or fruit.

In the region of unglaciated areas already discussed there are approximately 90 endemics, and the striking feature about their endemism is that in the majority of cases their nearest relatives are 1500 to 3000 miles away; such species as *Arenaria marcescens* Fernald (map 5), *Carex misandroides* Fernald (map 6), and *Puccinellia macra* Fern. & Weath. (map 7). Like many endemics discussed by Willis, these are highly localized species; but it is most difficult to imagine that they are newly evolving species, averaging 2000 miles away from their allies, and younger than the plants of the closely adjacent regions which have come into Gaspé and Newfoundland since the close of Pleistocene glaciation and which have there shown no pronounced departures from type.

Further to clarify this point, let us examine a flora which is wholly post-Pleistocene in some definite area. Southwestern Nova Scotia was denuded by the Pleistocene ice and has received its entire flora in post-glacial time; first, from the south *via* the continental shelf; second, from the north *via* New Brunswick. The element from the southern coastal plain isolated in Nova Scotia, often from Cape Cod, sometimes from New Jersey, and occasionally from the Carolinas, and in most instances comprising species



MAP 4. Range of *Schizaea pusilla* Pursh.

MAP 5. Ranges of *Arenaria obtusiloba* (Rydb.) Fernald (solid), and of *A. marcescens* Fernald (in ellipse).

MAP 6. Ranges of *Carex petricosa* Dewey and *C. Franklinii* Boott (Rocky Mts.), and of *C. misandroides* Fernald (in ellipse).



characteristic of the much warmer silicious belt from Florida to Alabama or Mississippi, has in Nova Scotia a single endemic species, a weed-like annual of a genus (*Agalinis*) in which all but specialists upon the group find the characters perplexingly variable. Otherwise, the best Nature has been able to do since Pleistocene time with species of the warm southern coastal plain isolated in Nova Scotia is to leave them unchanged or to increase the length of trichomes. If, then, in the extreme climate of Atlantic Nova Scotia Nature has not been able since the Pleistocene to evolve species out of the comparatively youthful stocks from the Tertiary sands of the South, it would be ridiculous to argue that the endemic species of the unglaciated regions of Gaspé and Newfoundland are just evolving and that their endemism is due to their excessive youth. As a matter of fact, their endemism, like the endemism of a large proportion of real species and real genera, is due in part to their isolation, in part to their great age.

I am perfectly aware that this is an old-fashioned doctrine, but it is a conclusion independently reached through close study of various floras through a period of a third of a century; and, I may add, my colleague, Professor Robinson, and every other experienced monographer with whom I have talked strongly reinforce the conviction that the best species, *i.e.*, those with the most stable and fundamental characters, are the old and commonly the isolated ones. Naturally there are very many good genera and species endemic to geologically young regions, such, for instance, as *Orontium*, *Polygonella*, *Sarracenia*, *Lechea*, *Rhexia*, *Sabatia*, *Bartonia*, *Sclerolepis*, etc., endemic to or radiating from the Tertiary sands of the Atlantic coastal plain of North America. These are all endemic genera of a very young region of eastern America; yet there is absolutely nothing in their distribution which indicates that they are a single day younger or a single day older on the coastal plain (or at its margin) or have there covered either a larger or a smaller area than *Psilocarya* (map 8), *Xyris*, *Eriocaulon*, *Centunculus*, *Lilaeopsis* (map 9), and numerous other genera of the coastal plain which have a broad range, especially over the southern hemisphere.

Thus we are led directly to consideration of another of the offspring of Age and Area, namely "Size and Space" or "Antiquity and Amplitude." Willis' statement is:

Keeping to the same circle of affinity, the larger families and genera will be the older, and will therefore occupy the most space. This, however, involves a break with the long current idea, that the larger families and genera are the successful ones, the smaller the (comparative) failures ("Age and Area," 113).

Or again, he says, more concisely: "the larger a genus, the older will it be, *within its own circle of affinity*."

Let us examine, then, the largest genera of dicotyledons in temperate North America. The list of the largest genera (with 100 or more species or near-species) in our flora is given in table 3.

TABLE 3. *Largest Genera of Dicotyledons in Temperate North America*

Eriogonum (150-200 species)	Gilia (107)
Crataegus (100-500)	Phacelia (114)
Rubus (?)	Pentstemon (100-150)
Potentilla (100 +)	Solidago (100-150)
Lupinus (100 +)	Aster (200-300)
Astragalus (250-350)	Erigeron (100-200)
Euphorbia (100-150)	Artemisia (23-106)
Oenothera and segregates (100 +)	Senecio (?)

With the American authority on Senecio taking part in our conference, I refrain from discussing that genus, and, on account of the wholly rudimentary state of our knowledge of Rubus and Crataegus, I will not take your time in a discussion of those mazes of perplexity, but the other genera are sufficiently understood so that we may examine their relation to Age and Area and their child Size and Space (or Antiquity and Amplitude). I have assumed that, unless more recent and authoritative treatments are at hand, the classification of the groups to which these genera belong may be taken from *Die natürlichen Pflanzenfamilien* or from the treatments in *Das Pflanzenreich*. I have therefore compiled lists of the complete "circle of affinity" of each genus and the number of species and the geographic range of each genus in the "circle."

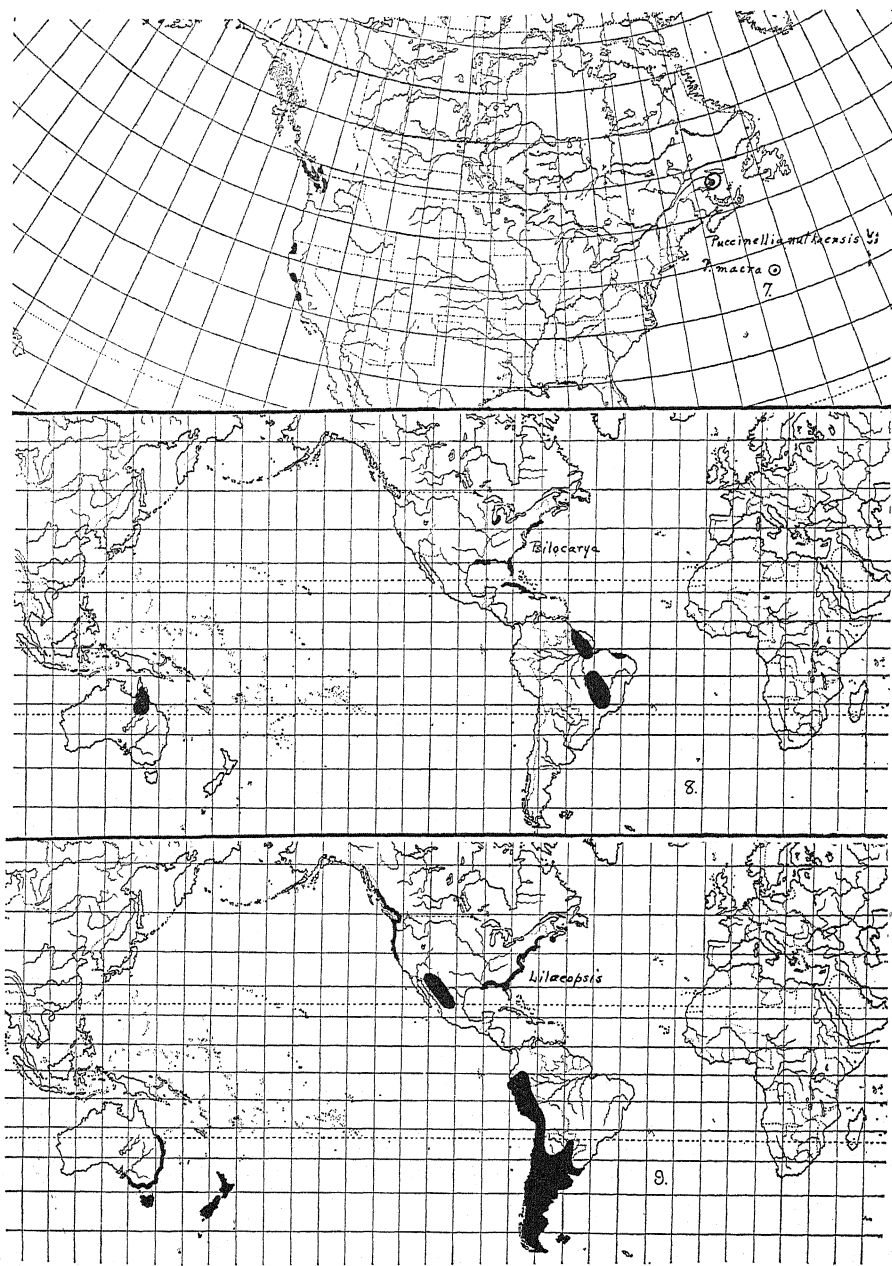
Thus the tribe Euphorbiae has 10 genera (the magic number upon which all deductions following Age and Area should be based): 6 with a total of 15 species (average, 2.5) in tropical Africa, Madagascar, and Bourbon; 1 with 2 species in West Australia; 1 with 1 species on the Balkan Peninsula; 1 with 15 species confined to tropical America; and the vast genus Euphorbia (600 species), world-wide in warm regions, with its tremendous development in the Mediterranean region, southwestern Asia, Africa, and the southwestern United States and Mexico. It has only 18 species in Australia and a single sea-shore representative in New Zealand.

Or take the Asterineae: 10 genera with a total of 36 species (average, 3-4) confined to North America; 5 genera with a total of 21 (average, 4) species confined to South America; 2 with 2 (average, 1) confined to the Mediterranean region; 1 with 50 widespread over Africa; 3 with 8 (average, 2.6) confined to central and eastern Asia; 4 with 25 (average, 6) confined to South Africa; 6 with 137 (average, 23) confined to Australia and New Zealand; 1 with 1 confined to Madagascar; 2 with 4 (average, 2) confined to St. Helena; 1 with 7 confined to Hawaii, and 2 with 13 (average, 6.5) sharing the Australasian region and southern South America. Besides these 37 small and highly localized genera (20 of which are endemic to remote areas of the southern hemisphere) with an average of 8 species each, there are 2 great genera: Aster, with 300-400 species, and Erigeron, with 150-200. More than half the species of these genera belong in temperate western North America, with the remainder chiefly in eastern North America, a few in South America and Eurasia, 5 anomalous Asters in South Africa, and 5 Erigerons in the Australian region.



Most of the other *largest* American genera give similar results: *Eriogonum*, with 150-200 species centering in the arid region of western America, but its "circle of affinity" containing the monotypic *Koenigia* which encircles the globe; *Crataegus*, confined to the northern hemisphere, but its "circle of affinity" containing numerous small Asiatic genera and the monotypic *Osteomeles*, endemic to Pacific islands; *Potentilla*, with more than 300 species, confined to the northern hemisphere with the exception of a single species of the essentially maritime subgenus *Anserina*, which has reached Australia and New Zealand; *Astragalus*, with 1300 species, 800-900 of them confined to the region of the Mediterranean and southwestern Asia and 200-300 to western North America, the genus completely wanting in Australia and New Zealand; and so on through the entire list.

Now, by Willis' interpretation these large genera are large because they are old, the small genera small because they are young. Naturally there is no *absolute* proof of this or of any other hypothetical proposition. But does not the whole matter leave the realm of hypothesis and enter the realm of demonstrable fact when we consider the geological history of the regions involved? It is generally conceded, I believe, that the most ancient living floras are now concentrated largely in Australia, New Zealand, and South Africa. It is also generally admitted that the Mediterranean basin, including a broad belt of North Africa and the country across Egypt and Syria, and, again, the great Austro-Russian basin extending southeastward to Persia were Tertiary sea bottom; similarly, by far the greatest part of the prairies, plains, and arid basins west of the Mississippi owe their present condition to Tertiary and even more recent geological activities. In other words, these regions were largely unavailable to their present floras millions of years after most of the groups which now distinguish Australia and New Zealand were at the height of their development. If, then, the largest genera of our flora, which are also overwhelmingly the largest genera of their own "circles of affinity," are, as Willis maintains, large because they are the ancient genera, is it not singular that *Eriogonum*, *Crataegus*, *Rubus* § *Eubatus*, *Lupinus*, *Astragalus*, *Oenothera*, *Gilia*, *Phacelia*, *Pentstemon*, *Solidago*, *Aplopappus*, and *Artemisia* should have not even a single species associated with the ancient types which inhabit Australia and New Zealand, and that only 0.02 of the total species of *Potentilla*, *Euphorbia*, *Aster*, and *Erigeron* should have reached those lands? Is it not equally noteworthy that, with the exception of *Crataegus* and the blackberries (*Rubus* § *Eubatus*), all the *largest* genera of the temperate North American flora should be centralized upon the Tertiary sea bottoms (relatively youthful country) of the Mediterranean and Austro-Russian basins of the Old World and in the vast area of youthful country west and southwest of the Mississippi? The blackberries are most virulent in central and western Europe, a region available to plants only since the Pleistocene glaciation, but likewise in the glaciated region and the Tertiary coastal plain of eastern



MAP 7. Ranges of *Puccinellia nutkaensis* (Presl.) Fern. and Weath. (Pacific coast), and of *P. macra* Fern. and Weath. (in ellipse).

MAP 8. Range of the genus *Psilocarya*.

MAP 9. Range of the genus *Lilaeopsis*.

North America. *Crataegus* has its phenomenal development in the eastern United States and southern Canada, where it must have produced a multitude of its species in post-glacial time.

It should be perfectly obvious that these genera and likewise such overwhelmingly large genera of Europe and southwestern Asia (but not of Australia and New Zealand) as *Dianthus* (200-300 Eurasian species), *Silene* (300-400), *Verbascum* (150-200), *Cousinia* (150), *Centaurea* (200-300), and *Hieracium* (600-700) are really very young, or, if geologically old (*Crataegus* and *Rubus* for example), they have been encouraged by modern conditions to rapid multiplication. And if we estimate success of plants by their ability to cover country, to take care of themselves, and to multiply their variations to the bewilderment of the best systematists, then these are surely successful genera. No society for the protection of native plants has thought it necessary to urge protection for *Eriogonum*, *Crataegus*, *Rubus* § *Eubatus*, *Euphorbia*, *Solidago*, *Artemisia*, *Centaurea*, and *Hieracium*; but there are plenty of people (including many botanists) who would be glad to have them suppressed.

The genera of eastern America which need scrupulous protection are the woodland herbs of the Alleghenian forest, the genera which we largely share with Japan and China and which are consequently among the oldest plants now living on the North American continent. These are chiefly diatypes, with one species in eastern America, one in eastern Asia, though some of the genera have more than two species; but, while the systematist shrugs his shoulders and waves you away if you bring him for determination a collection of the youthful species of *Lupinus*, *Oenothera*, *Aster*, or *Artemisia*, he can give a prompt and explicit determination of *Caulophyllum*, *Podophyllum*, *Dicentra*, *Panax*, *Cryptotaenia*, *Epigaea*, *Chiogenes*, and *Mitchella*—and why should he not? These are genera of the eastern Asiatic-eastern American forests; and they are absolutely segregated genera with no complications of freely intergradient species, not, as Age and Area asserts, because they are young beginners but because they are old veterans.

Let us turn now for a moment to the largest genus of New Zealand. This is what Willis, following the most extreme of British conservative treatments, still calls *Veronica*; and because he calls it *Veronica* he takes it to be congeneric with the world-wide but chiefly Eurasian *Euveronica*. It is thus natural for him to conclude that the largest genus in New Zealand is cosmopolitan (Age and Area, 165). *Euveronica*, however, is a group of herbaceous or subherbaceous plants with marcescent leaves, with corolla usually 4-lobed, and with capsules laterally compressed and loculicidally dehiscent. Only 13 species of *Euveronica* are enumerated by Cheeseman from New Zealand. Then in New Zealand there is, included by Cheeseman and by Willis under *Veronica*, the endemic genus *Pygmaea* Hook. fil., with closely imbricated tiny chartaceous leaves, terminal and solitary flowers with the corolla normally 5-lobed, and turgid or dorsally compressed

capsules. But the great bulk of New Zealand "Veronicas" are shrubs and trees with the coriaceous leaves disarticulating and leaving prominent scars, the capsule turgid and dorsally (not laterally) compressed, and the dehiscence septicidal. In other words, the famous woody "Veronicas" of New Zealand belong to the genus *Hebe* of Commerson. *Hebe* is confined to the Australasian region and to the Falkland and Fuegian region, and, even though it and *Pygmaea* are by some botanists crowded into *Veronica*, their long-known morphological differences, associated as they are with pronounced regional development, cannot be waved aside as trifling matters. *Pygmaea* and *Hebe* are wholly different groups from *Euveronica*, and it seems fairly clear that the largest genus of New Zealand shows its antiquity, not through its cosmopolitan distribution, but through the fact that this essentially woody group is now confined to two remote regions which have presumably not been connected since the destruction of ancient Antarctica. This of course does not mean that the scores of living and "difficult" species of *Hebe* in New Zealand are old. We have a similar multiplication of modern species and varieties in many old genera of the northern hemisphere: *Salix*, *Carya*, *Betula*, *Quercus*, *Crataegus*, *Rubus*, *Tilia*, *Rhododendron*, and others which date chiefly from Cretaceous time. It simply means that under favorable conditions ancient generic stocks may enter a cycle of rapid multiplication of species.

This finishes the major points which I shall have time to discuss, but one other matter must be noted. This is the character of some of the statistics used by Willis in attempting to prove his points. For instance, in arguing that there are more endemics in tropical than in temperate regions, he makes what would seem a clinching assertion: "North-temperate America has perhaps 400, but Ceylon alone has 800 endemics, and Brazil perhaps 12,000" (p. 89). In view of the 1000 endemic grasses recognized in temperate North America, the 400 *Carices*, the more than 300 *Cruciferae* or *Umbelliferae*, the full 100 *Malvaceae*, to say nothing of *Eriogonum*, *Crataegus*, *Astragalus*, *Euphorbia*, and all the rest, the utter folly of attempting to prove anything by such wholly unreliable statistics must be apparent. To those familiar with the literature of Age and Area the source of Willis' statement that there are only "perhaps" 400 endemic species in temperate North America is obvious. The citations in the chapter clearly indicate that he was trying to quote from Sinnott's statement that "400 genera were recorded as being endemic or essentially so in temperate North America" (*Am. Nat.* 50:468); but to count the endemic genera of temperate North America against the endemic species of Brazil is fallacious, for it sadly mixes different categories; and every instance of inability to quote correctly or to differentiate clearly between unlike groups naturally weakens the confidence which can be placed in the conclusions.

In summary I may say that, in as far as my own studies of the floras of the northern hemisphere indicate, Age and Area is of little service. In

fact, much of the evidence in the region with which I am most familiar runs absolutely counter to this hypothesis. If, as I at first stated, the world were absolutely static, Age and Area would supply an obvious working principle; but the greatest land masses of the world have not been static, and the resulting dispersal of floras is most complex. In view of the great influence of the historic and an endless series of edaphic and other factors, our studies should be directed toward a clear elucidation of these positive forces and above all to a thorough knowledge of the plants themselves. Until we know our plants and the complex factors to which each of them and the various associations of them are responding, we can make little progress in the satisfactory working out of universal laws.

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## AGE AND AREA AND THE HISTORY OF SPECIES<sup>1</sup>

EDMUND W. SINNOTT

It is difficult to evaluate a scientific hypothesis properly until time enough has elapsed for its mature consideration from all points of view. Particularly in such a case as Dr. Willis' hypothesis of Age and Area, which touches so many fields of biology, it is essential that specialists in these various fields pass judgment upon it before we can estimate its strength or weakness. During the eight years in which it has been before the biological world such judgments have been accumulating, and the time is therefore near when we may reasonably expect to arrive at a just and well considered verdict as to the usefulness of this particular hypothesis.

Dr. Willis' original suggestion was that, as a general rule, the extent of range of a species may be used as an indication of the age of that species. As necessary corollaries of this idea he maintains that the "dying out" of species occurs but rarely, and that new forms arise by mutation rather than by local adaptation through natural selection. To meet various criticisms, the hypothesis has latterly been hedged about and modified by numerous restrictions and provisos which need not be discussed here, although the question may well be raised as to whether they do not rather effectually neutralize the hypothesis itself and render the operation of mere age a very minor one in determining the area over which a species is at present distributed. The bearing of the hypothesis in its present form on the problems of plant geography, endemism, the origin of species, and questions related thereto have been adequately discussed by previous speakers in this symposium and in numerous papers by others. Interest has been focused so closely upon these matters that insufficient attention has perhaps been devoted to other important implications of the theory. Instead, therefore, of attempting to discuss, or even briefly to summarize, the whole question, the author proposes here to consider one of these other implications—the bearing of age and area upon our ideas as to the normal course of the history of a plant species, a problem distinct in many ways from that of its origin or its environmental relationships. Students of the evolutionary drama have approached this problem from various points of view and have built up a rather definite picture of the birth, life, and death of a typical species. If the hypothesis of age and area is inconsistent with this, either the picture must be radically redrawn or the hypothesis itself must fall. At no point, perhaps, are we at present better able to test the soundness of Age and Area than in this, its historical implication.

<sup>1</sup> Read in the symposium on "The Age-and-area Hypothesis" at the meeting of the Systematic Section of the Botanical Society of America at Cincinnati, December 29, 1923.

Dr. Willis' conception of the history of an ordinary species is that it has its origin, gradually extends its range as time goes on, and ultimately becomes widely dispersed, in which state, barring accidents, it has attained essential permanence. Does this agree with the results of other lines of evolutionary investigation?

Evidence of value in the solution of such a problem should be drawn from as wide a range of sources as possible. Dr. Willis' method is almost entirely statistical, and in this respect is in line with other laudable attempts to treat the facts of biology in a quantitative rather than entirely in a qualitative manner. The obvious disadvantage of all such statistical analyses, however, when unsupported by other data, is that the individual instance, with its particular antecedents and consequences, is lost in the mass. Conclusions drawn from such a study may sometimes lead to useful predictions, but often fail entirely in explaining the actual causes underlying the phenomena investigated. Dr. Willis' treatment of his data need not be discussed further here except to say that his figures may often be interpreted and explained in several different ways.

But even granting that conclusions based on such a statistical analysis, unsupported by other evidence, may not always be sound, have we any reason to believe that in our present problem they are incorrect?

The most obvious and conclusive source of information in all such historical questions is evidently the fossil record itself. This has already been discussed by a previous speaker and has always formed one of the most serious stumbling-blocks to an acceptance of the age-and-area hypothesis. To one who is familiar with the tremendous pageant presented by the evolution of the vegetable kingdom and who has seen group after group arise, flourish, and disappear, the suggestion that the dying out of species is a comparatively rare phenomenon carries little conviction. As he goes back in the fossil record, he finds that one by one the species and genera now familiar do disappear and that strange forms occupy their places. Where, he asks, are now those thousands upon thousands of species which once covered the earth? And why is it that as a result of this enormous evolutionary productivity there are not hosts of widely dispersed, presumably ancient forms still alive, instead of the handful which he actually finds? That the disappearance of these species has always been due to climatic modifications or other environmental changes may be true, as Dr. Willis asserts, but the fact that there has been such wholesale dying out in the past renders it highly probable that species are also disappearing today, and persistently raises the question as to whether many ranges are not stationary or even dwindling rather than invariably expanding, as Age and Area demands. In the case of species and genera which we know from fossil evidence to be occupying smaller ranges than in the past, Dr. Willis maintains that we must include this fossil range with the present one in determining the age of a species; but for the practical usefulness of the



hypothesis this is a very unsatisfactory solution, since how are we to know (except in rare cases) which forms have once had a wider range and just how wide this range was?

Chiefly on the basis of fossil evidence, the idea has frequently been proposed that species, and larger organic groups, have a definite "life history," somewhat comparable to that of an individual. They are born, reach in time the height of their vigor and success, give off a group of new forms, and finally become extinct. This hypothesis is of course very difficult to prove, but we must admit that the geological data, as far as they go, agree much more closely with such a conception of specific history than with the one which is necessarily forced upon us if we adopt Dr. Willis' hypothesis.

Aside from evidence derived from fossils, however, we can obtain a good deal of information as to the history of plant types directly from the distribution of living species in the world today. Shortly after the promulgation of the age-and-area hypothesis, the writer<sup>2</sup> called attention to one aspect of the problem of endemism which it did not satisfactorily explain—the fact that in more or less isolated regions, including those especially studied by Dr. Willis, there are a considerable number of genera which are themselves not endemic but which contain *only endemic species*. Such, for example, in the flora of Ceylon are *Dipterocarpus*, *Shorea*, *Hopea*, *Xylopia*, *Euonymus*, *Gymnostachyum*, *Actinodaphne*, *Lasianthus*, *Mangifera*, *Semecarpus*, and many others, sixty-three in all, constituting eight percent of all the dicotyledonous genera. In New Zealand the case is even more striking, for here there are ninety non-endemic genera of dicotyledons, or forty-three percent of the whole, which are represented here only by endemic species, and among these are some of the commonest and most important genera of the islands. What can have been the history of these genera? According to Dr. Willis' assumption, the original representative of each of these (and of all other) genera in the regions in question was some wide-ranging species which came in from the outside; and all the endemic species have arisen from this original invader, by mutation or otherwise. But in these particular genera, which constitute a very considerable portion of the floras in question and therefore can not in any sense be said to be "lost in the crowd," where now is the original invading species? It assuredly ought to be here as the commonest and most widespread member of the genus. Something disastrous must have happened to it, for at present there is no species in any of these genera which is found beyond the limits of the region, although the genera themselves are widely dispersed and presumably originated elsewhere. The original species in such cases has either been exterminated, which seems unlikely in view of the success of its offspring, or has been "swamped" out of existence by interbreeding with the various local types which have arisen from it in its new and isolated environment. It is perhaps noteworthy that

<sup>2</sup> Sinnott, E. W. The age-and-area hypothesis and the problem of endemism. *Annals Bot.*, 31: 209-216. 1917.

the more isolated and ancient the flora is, the larger is the proportion of these genera which are non-endemic but contain only endemic species. Their existence, particularly in such large numbers, has proven very difficult for Dr. Willis to explain, and his reply to the objection which they raise against the hypothesis is perhaps the least convincing of his various answers to criticism.

Let us endeavor to reconstruct the various steps which apparently took place in the history of such a group. A widespread and successful species crosses over to an island from the mainland, we will say, and there establishes itself. Its members are cut off from the rest of the species and subjected to a somewhat different environment. They perhaps interbreed frequently with new forms which have arisen from among their number. What more natural than that they should slowly change their type and in time lose specific identity with individuals of the old stock on the mainland? The species has not "died out,"<sup>3</sup> in the sense of extinction, but has become transformed, and a whole new group of local species has thus arisen. Nor has such transformation occurred only where the species has invaded an island. In every portion of its range where a group of individuals has become isolated from the rest, even to a slight degree, it will tend to develop a local race or races; and such groups will become more and more numerous as the species extends itself over a wider and wider range. The student of plant and animal distribution is acquainted with numberless instances of local groups of species or varieties, almost certainly descended from some one original form, but now changed so radically that they are no longer identical with anything elsewhere. As to whether this change has been accomplished in the manner suggested by Lamarck, by Darwin, or by De Vries is another problem; but that, given sufficient time, isolation is almost inevitably accompanied by change in type will be generally admitted.

This picture of the normal history of a species agrees in its essential respects with that presented by fossils, and is sharply at variance with the assumptions of Age and Area. The species originates in a given spot—how or why we may for the moment disregard—and gradually extends its range. The possible limits reached by one species will be wider than those reached by another, but we will admit that, up to a certain point, other things being equal, the longer the species exists the wider its range will tend to be. "Up to a certain point" is an important proviso, however, for when the range once begins to be rather extensive and groups of individuals become widely separated, the disintegrative influence of isolation begins to make itself felt, and the single widely dispersed species gives rise to peripheral groups of new forms and ultimately becomes itself so changed as to alter its specific character. Indeed, among the higher plants, where evolutionary change is

<sup>3</sup> It may be mentioned parenthetically that a sharp distinction should be drawn (which Dr. Willis does not do) between the dying out which is due to actual extermination and that which is due merely to specific change.

most rapid, it is doubtful whether a species can long maintain itself unaltered over a wide area. At any rate, few seem to do so, and among these few critical study frequently discloses numerous local varieties. This is perhaps one of the most important reasons for the fact brought out by Dr. Willis that local species are relatively very numerous and widely ranging ones are progressively rarer and rarer. Of course we should remember that only the exceptional species is capable of spreading over a range wide enough so that the disintegrative effects of local isolation are very marked. Most species, either through lack of adaptability or because of barriers, are limited to a comparatively narrow range and are therefore more likely to maintain their specific integrity.

But if it be true that a species can not long maintain itself intact over a wide area, it must follow that those species which today do have wide ranges are comparatively young types which have just reached the pinnacle of their success and have not yet had time to become broken up into new, local forms. This, of course, is precisely the reverse of Dr. Willis' contention.

Now it may be objected that this does not affect the fundamental position of Willis and that the widespread species are necessarily older than the local types to which they gave rise. The fact that the parent species are gradually disintegrating into smaller ones, however, and that the range of these parents must therefore be shrinking or breaking up, introduces a considerable element of doubt into an interpretation of range as a criterion of age. Furthermore, in the practical application of such an hypothesis as Age and Area to the history of a given flora, it is knowledge as to the relative antiquity of genera and larger plant groups, the various elements of which the flora is composed, which is often desired. For such a study, the conception here reached is diametrically opposed to that of Age and Area. In an insular flora, for example, those species which are not endemic and which are thus identical with mainland forms are not to be regarded as very ancient members of the flora, as Dr. Willis maintains, but rather as relatively recent arrivals which have not yet had time to lose their specific identity with their parent forms on the mainland. True, each of these species may have given rise to a group of local endemics which are thus younger than itself, but such a group is evidently a younger element in the flora than a non-endemic genus in which the original invader has disappeared; as this, in turn, is less ancient than an endemic genus. Endemic types (even if we exclude relict endemics, most of which are undoubtedly old) are therefore to be looked upon in general as relatively ancient elements in isolated floras rather than as relatively recent ones, as Dr. Willis maintains.

We may therefore conclude as a result of the evidence at hand that the picture of the normal history of a species which is presented by the age-and-area hypothesis, and that which may be drawn from a study of the geological record and of the effects of isolation, are markedly different. According to the former, the species spreads slowly from its point of origin and with the

passage of time gradually reaches the maximum range of which it is capable. Here it remains in its old age, a widely dispersed, ancient type which will persist thus indefinitely unless there occurs some unfavorable change in the environment. According to the latter view, however, the species, after spreading from its center of origin and reaching the limit of its possible extent, does not there maintain itself indefinitely but tends instead, especially if it has covered a very wide range, to break up, soon after its climax, into a group of new species and thus finally to lose its specific identity. A species may thus be likened to a wave, spreading in all directions from its point of origin and ultimately breaking up into a series of new wavelets. These, in turn, go on and finally become themselves disintegrated. Wave after wave of species has thus spread and broken during the course of evolution, each losing itself in its descendants and thus gradually building up the plant kingdom as we know it today. This is the picture of the evolutionary history of species which best agrees with the evidence at hand from all sources, and the fact that the age-and-area hypothesis, with its implication of ultimately static species, does not accord with it is one of the most serious objections which may be raised against the validity of the hypothesis in general.

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# INFLUENCE OF ENVIRONMENTAL FACTORS ON THE INFECTION OF SORGHUMS AND OATS BY SMUTS II. EXPERIMENTS WITH COVERED SMUT OF OATS AND GENERAL CONSIDERATIONS<sup>1</sup>

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In a previous publication the writers (38) have described their experiments to determine the influence of environmental factors on the infection of sorghums by covered and loose kernel smuts. The present paper takes up a similar series of experiments on the infection of oats by *Ustilago levis* (K. & S.) Magn. The methods employed were essentially the same as described for the previous studies.

In 1922 a preliminary test was made to determine the influence of moisture on the infection of *Avena nuda* L. var. *inermis* (S. N. 30) by both *Ustilago avenae* (Pers.) Jens. and *U. levis* (K. & S.) Magn. The experiment was planned primarily to determine whether sand would be a suitable substratum for carrying out an extended series of experiments on the influence of temperature, moisture, and other factors on infection. The sand used passed through a 20-mesh sieve but not through a 40-mesh sieve, and had a reaction of pH 7.2. The seeds were germinated in the laboratory at a temperature of 19°-22° C. Four soil moistures were used—20, 40, 60, and 80 percent. The results are given in table I.

TABLE I. Influence of Moisture on Infection of *Avena nuda* L. var. *inermis* (S. N. 30) by *Ustilago avenae* and *U. levis*, 1922; Laboratory Temperature, 19-22° C.; Sand, pH 7.2

% Moisture	<i>Ustilago avenae</i>			<i>Ustilago levis</i>		
	No. Plants	No. Inf.	% Inf.	No. Plants	No. Inf.	% Inf.
20	31	12	38.7	24	21	87.5
40	26	4	15.3	29	16	55.1
60	29	2	6.8	23	8	34.7
80	16	1	6.2	21	5	23.8

In the series with *Ustilago avenae*, the highest infection (38.7 percent) was obtained in the 20-percent moisture. There was a marked decrease in the amount of infection in 40-percent moisture and a still further falling off in both 60- and 80-percent moistures. The highest percentage of infection with *Ustilago levis* (87.5 percent) also was obtained in the 20-percent

<sup>1</sup> Brooklyn Botanic Garden Contributions no. 37.

moisture. There was a gradual decrease in the amount of infection in the moistures of 40, 60, and 80 percent. The highest infection with both smuts was obtained in the lowest moisture with a decrease in the amount of infection as the moisture was increased.

A more extensive series of experiments on the influence of moisture and temperature was carried out in 1923. However, only the covered smut, *Ustilago levis*, was used for inoculating two very susceptible varieties, *Avena nuda* L. var. *inermis* (S. N. 30) and *A. sativa* L. var. Victor (S. N. 126).

#### TEMPERATURE AND MOISTURE SERIES

The cultures were grown in the constant-temperature tank at temperatures of 5°, 10°, 15°, 20°, 25°, and 30° C., and in sand moistures of 15, 20, 25, 30, 35, 40, 50, and 60 percent of the water-holding capacity. After the emergence of the plants from the sand they were transferred to the field. The temperatures were very accurately controlled. It was not possible to secure definite sand moistures. Some variation occurred, and considerable difficulty was encountered in the low moistures at the high temperatures. Some, at least, of the irregularities of the curve may be accounted for by variations from the sand moisture indicated. The results, which are given in tables 2 and 3 and illustrated graphically in figures 1 and 2, are to be analyzed on the basis of both temperature and moisture relations.

#### Results with *Avena nuda*

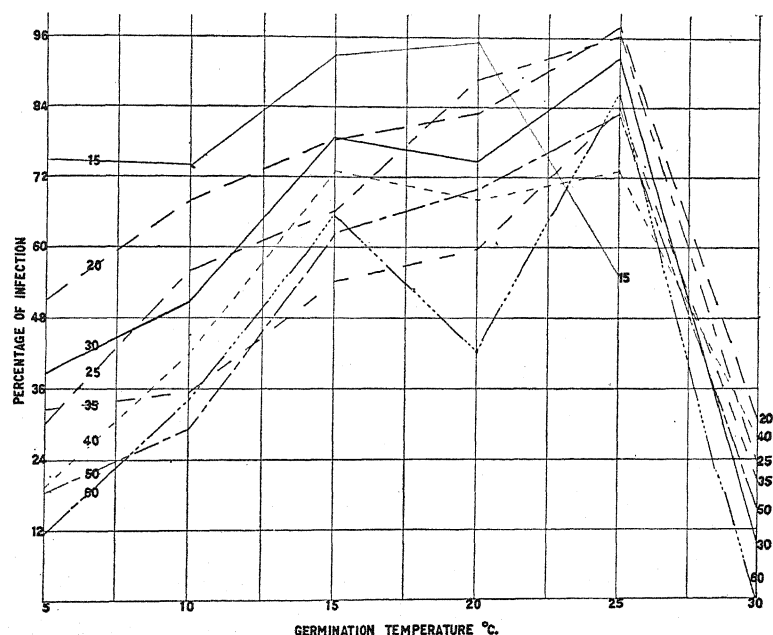
An examination of the table shows moderate to high infections of *Avena nuda* at 5° C. in all moistures. While there are slight irregularities, the percentages of infection decrease from the highest infection (75 percent) in 15-percent moisture to a minimum of 11.7 percent infection in the 60-percent moisture.

TABLE 2. Influence of Temperature and Moisture on Infection of *Avena nuda* L. var. *inermis* (S. N. 30) by *Ustilago levis* (K. and S.) Magn., 1923

% Moisture	5° C.		10° C.		15° C.		20° C.		25° C.		30° C.		Total	
	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.
15	28	75.0	54	74.0	54	92.6	21	95.2	56	55.3	—	—	213	76.0
20	33	51.5	56	67.8	56	78.5	41	82.9	49	97.9	96	30.2	331	63.4
25	36	30.5	57	56.1	59	66.1	54	88.8	57	96.6	103	23.3	366	57.1
30	39	38.4	59	50.8	52	78.8	55	74.5	103	92.2	99	15.1	407	58.2
35	43	32.5	51	35.2	59	54.2	56	58.9	50	84.0	40	20.0	299	49.1
40	47	19.1	50	42.0	60	73.3	56	67.8	67	73.1	43	27.9	323	53.5
50	54	18.5	48	29.1	53	62.2	51	70.5	90	83.3	104	15.3	400	46.0
60	34	11.7	58	34.4	55	65.4	57	42.1	104	86.5	86	0	394	44.1
	314	32.1	433	49.1	448	71.2	391	70.0	576	84.2	571	18.2	2,733	54.7



The percentages of infection at 10° C. are higher in practically all moistures than at 5°; the only exception was in the lowest moisture in which the percentage of infection was 74 percent. The lowest percentage of infection (29.1 percent) was obtained in the 50-percent moisture.



TEXT FIG. 1. Influence of soil moisture and temperature on infection of *Avena nuda* L. var. *inermis* (S. N. 30) by *Ustilago levis*. The lines in the graph are designated by figures indicating the percentage of moisture: 15, 20, 25, 30, 35, 40, 50, and 60 percent.

Much higher percentages of infection were obtained in every moisture at 15° C. than at 10°. The highest percentage (92.6 percent) occurred in the lowest moisture. While there were some irregularities, in general there was a decrease in the percentage of infection as the amount of moisture was increased. However, the lowest infection (54.2 percent) occurred in the 35-percent moisture.

The percentage of infection at 20° C. in most moistures was higher than at 15°. The highest percentage of infection (95.2 percent) occurred in the lowest moisture and the lowest infection (42.1 percent) in the highest moisture.

The percentages of infection at 25° C. were greater in all moistures, except the lowest, than they were at 20°. The highest infection (97.9 percent) occurred in the 20-percent moisture and the lowest (53.5 percent) in the 15-percent moisture.

There was a very decided decrease in the percentage of infection at 30° C. No plants were grown in the 15-percent moisture. The amount of



infection varied somewhat irregularly with the percentage of moisture; the highest (30.2 percent), however, was obtained in the 20-percent moisture. Negative results occurred in the highest moisture.

It is evident that *Avena nuda* was readily infected by *Ustilago levis* over a wide range of temperatures—5° to 30° C. The highest infections in general were obtained at a temperature of 25°. These, however, depended upon the soil moisture. Infection also took place over a wide range of moistures, but low soil moistures were much more favorable for high percentage of infection than high soil moistures. The highest infections were secured in the 15-percent moisture at 5°, 10°, 15°, and 20°, and in the 20-percent moisture at 25° and at 30° C.

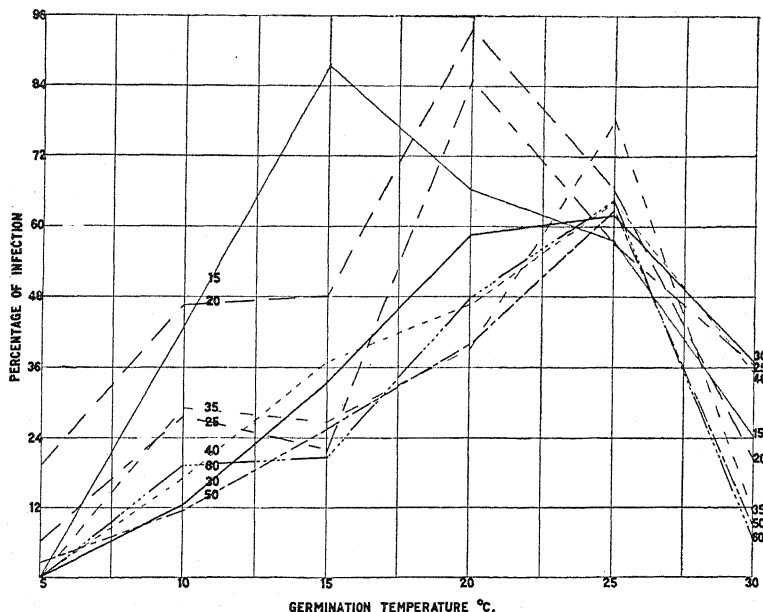
### Results with Victor

Infections with Victor were obtained in only three moistures (20, 25, and 50 percent) at 5° C. Infections were obtained in every moisture at 10°, the highest infection (47 percent) occurring in the 20-percent moisture and the lowest (10 percent) in the 50-percent moisture. The highest percentage of infection (87.5 percent) was obtained in the 15-percent moisture and the lowest (21.1 percent) in the 60-percent moisture at 15°. The percentages of infection at 20° generally were much higher than at 15°. In the lowest moisture, however, the percentage of infection was 66.6 percent, as compared with 87.5 percent at 15°. The highest infection (93.7 percent) occurred in the 20-percent moisture and the lowest (39.2 percent) in the 35-percent moisture. There was a decrease in the percentage of infection in the lower moistures at 25° as compared with 20°. On the other hand, there was an increase of infection in the higher moistures. The highest infection (78.2 percent) occurred in the 35-percent moisture and the lowest (57.9 percent) in the 25-percent moisture. There was a marked decrease in the percentage of infection at 30° in all moistures. The highest (36.7 percent) occurred in the 30-percent moisture and the lowest (6.9 percent) in the 60-percent moisture.

TABLE 3. Influence of Temperature and Moisture on Infection of *Avena sativa* L. var. Victor (S. N. 126) by *Ustilago levis* (K. and S.) Magn., 1923

% Moisture	5° C.		10° C.		15° C.		20° C.		25° C.		30° C.		Total	
	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.	No. Plts.	% Inf.
15	23	0	51	43.1	48	87.5	36	66.6	48	58.5	33	24.2	239	51.8
20	26	19.2	51	47.0	52	48.0	32	93.7	62	66.1	88	20.4	311	45.9
25	46	6.5	53	28.3	53	22.6	48	85.0	69	57.9	85	36.4	354	39.8
30	24	0	48	12.5	59	33.8	46	58.6	50	62.0	68	36.7	295	36.9
35	51	0	55	29.0	49	26.5	51	39.2	46	78.2	94	11.6	346	27.7
40	19	0	35	17.1	46	36.9	45	46.6	100	64.0	62	35.4	307	42.3
50	36	2.7	60	10.0	47	25.5	55	40.0	102	62.7	94	8.5	394	28.6
60	40	0	52	19.2	52	21.1	50	48.0	93	64.5	115	6.9	402	28.1
	265	3.3	405	25.9	406	37.4	363	57.3	570	63.8	639	20.5	2,648	36.5

The variety Victor in general gave the highest infection at 20° C. in the lower soil moistures, but at 25° in the higher moistures. The results showed that, except at the temperature of 25°, the low moistures were much more favorable for infection than high moistures.



TEXT FIG. 2. Influence of soil moisture and temperature on infection of *Avena sativa* L. var. Victor (S. N. 126) by *Ustilago levis*. The lines in the graph are designated by figures indicating the percentage of moisture: 15, 20, 25, 30, 35, 40, 50, and 60 percent.

A comparison of the results between *Avena nuda* and Victor indicate a much greater susceptibility of the former at 5° C. On the other hand, there is a greater susceptibility of Victor at 30°. *Avena nuda* is most severely infected at 25° in all moistures, except 15-percent moisture. On the other hand, Victor is most severely infected at 20° in the low moistures and at 25° in higher moistures. The highest infection obtained with Victor was 93.7 percent at 20° in 20-percent moisture. The highest infection of *A. nuda* was 97.9 percent at 25° and in the 20-percent moisture.

So far as moisture is concerned, the results compare very favorably with those obtained in 1922, giving definite evidence that low moistures are much more favorable than high soil moistures for the infection of oats by *Ustilago levis*. However, the temperature and moisture must be considered together, as they are interdependent factors.

An examination of the graphs shows clearly how the temperature most favorable for infection varies with the moisture content of the soil. It is particularly evident that for Victor 15° C. is the optimum, so called, in the

15-percent soil moisture; on the other hand, 20° C. is the optimum in soil moistures of 20 and 25 percent, and 25° is the optimum at still higher soil moistures. The junior author (9) also found that the temperature most favorable for infection of barley by covered smut varied with the soil reaction. In acid soils of two moistures—40 and 50 percent—the optimum temperature for infection was 20° C., while in neutral or alkaline soils the optimum temperature was 10° or 15° C. It can not be too strongly emphasized that the term "optimum temperature for infection" is without significance unless due regard is paid to other possible influencing factors.

#### INFLUENCE OF SOIL REACTION ON THE INFECTION OF OATS BY *USTILAGO LEVIS*

In 1923 *Avena nuda* (S. N. 30) and Victor (S. N. 126) were grown in sand with seven different soil reactions—pH 4.6, 5.0, 6.6, 7.4, 7.8, 8.4, and 8.6. The sand moisture was 20 percent and the temperature was 19°–22° C. The data are given in table 4 and illustrated graphically in figure 3.

TABLE 4. *Influence of Acidity on Infection of Oats by Ustilago levis, 1923; Laboratory Temperature, 19–22° C.; Sand, Moisture 20 percent*

pH	<i>A. nuda</i> (30)			Victor (126)		
	No. Plants	No. Inf.	% Inf.	No. Plants	No. Inf.	% Inf.
4.6	26	1	3.8	25	3	12.0
5.0	27	4	14.8	28	5	17.8
6.6	10	2	20.0	42	33	78.5
7.4	36	23	63.8	50	46	92.0
7.8	43	16	37.1	42	24	57.1
8.4	31	1	3.2	46	12	26.0
8.6	26	2	7.6	11	2	18.1

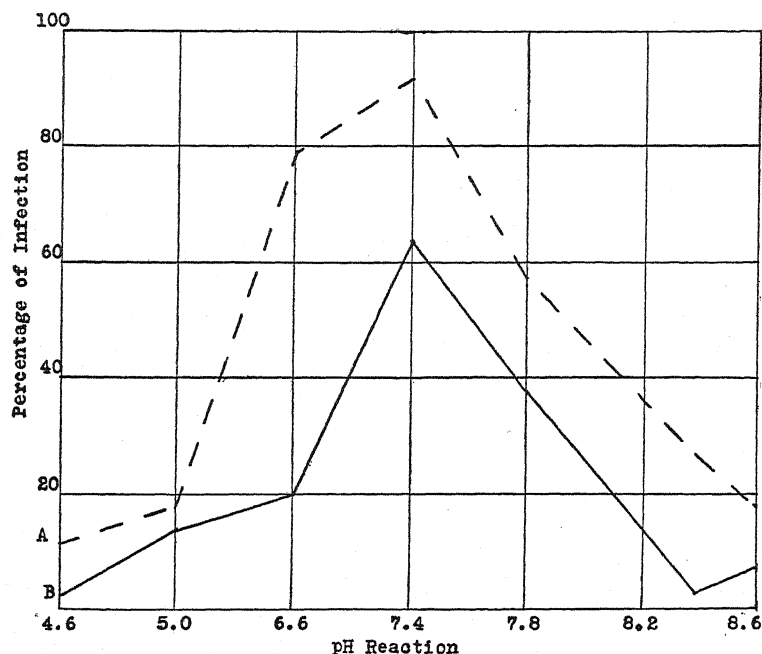
Infections of *Avena nuda* were obtained in all the experiments. The highest infection (63.8 percent) was secured in pH 7.4. There was a decrease in the amount of infection as the pH value was raised or lowered beyond pH 7.4, except that a slightly higher percentage of infection was obtained in pH 8.6 as compared with pH 8.4.

Victor gave the highest percentage of infection (92 percent) in pH 7.4. There was a regular decrease in the percentage of infection in higher and lower concentrations. The percentages of infection secured with Victor were higher than those with *A. nuda*.

#### GENERAL DISCUSSION

It is quite clearly established by the experiments described above that there is a definite connection between certain environal factors and the infection of their respective hosts by the covered smut of oats and the two

kernel smuts of sorghums. Other investigators have contributed evidence of a similar connection between various cereal smuts and their hosts.



TEXT FIG. 3. Influence of pH reaction on infection of oats by *Ustilago levis*. A, *Avena sativa* L. var. Victor (S. N. 126). B, *Avena nuda* L. var. *inermis* (S. N. 30).

*Influence of Date of Seeding on Smut Infection.* Observations have been made on the relation of the date of sowing oats to the occurrence of oat smut, generally *Ustilago avenae*. Kellerman and Swingle (23) found no obvious differences in the amount of smut on a number of different seedings made between March 22 and April 12. Jones (21) found a moderate amount of smut on oats sown May 4, but no infection occurred in the same varieties sown July 13. Rose (39) sowed 63 varieties on two different dates, and in general obtained higher percentages of infection in the later seeding. Clinton (7), Ravn (34), von Tubeuf (41), and Heald (10) found comparatively low percentages of infection in very early seedings, moderate to high infections in mid-season seedings, and a decrease in late seedings. Hiltner (15) found a progressive increase in the amount of smut in seven seedings from March 17 to May 21.

In 1922 the writers sowed separate sets of seeds of *Avena nuda* L. var. *inermis* (S. N. 294) and *A. sativa* L. var. Victor (S. N. 126), inoculated with spores of *Ustilago avenae* and *U. levis* on five different dates. The data are given in table 5.

TABLE 5. *Influence of Date of Planting on Occurrence of Smuts of Oats, 1922*

Species and Variety	Date of Seeding	<i>Ustilago avenae</i>			<i>Ustilago levis</i>		
		No. Plants	No. Inf.	% Inf.	No. Plants	No. Inf.	% Inf.
<i>Avena nuda</i> L. var. <i>inermis</i> (S. N. 294)	Mar. 29	616	139	22.5	520	456	87.6
	Apr. 6	159	138	86.7	164	161	98.1
	Apr. 20	138	6	4.3	118	116	98.3
	May 11	209	44	21.0	158	147	93.0
	May 31	105	1	.9	146	96	65.7
<i>Avena sativa</i> L. var. <i>Victor</i> (S. N. 126)	Mar. 29	164	36	21.9	216	87	40.2
	Apr. 6	223	91	40.8	207	118	57.0
	Apr. 20	107	14	13.0	112	63	56.2
	May 11	102	31	30.3	90	56	62.2
	May 31	74	3	4.0	46	6	13.0

*Avena nuda* L. var. *inermis* gave, as a rule, high percentages of infection. The highest percentage (86.7 percent) by *Ustilago avenae* was obtained in the planting of April 6. In the planting of April 20, only a very small percentage of plants (4.3 percent) was infected. The percentage of plants infected in the plantings of March 29 and May 11 was about the same. In the last planting, less than 1 percent infection was secured. The highest infection with *Ustilago levis* was obtained in the planting of April 20, although very high percentages were secured in the plantings of April 6 and May 11. A slightly lower percentage of infection occurred in the planting of March 29 and there was a marked decrease in the planting of May 31. *A. sativa* L. var. *Victor* gave the highest percentage of infection with *Ustilago avenae* in the planting of April 6. The planting of April 20 gave a rather low percentage of infection; that of May 11 somewhat higher. The highest percentage of infection by *Ustilago levis* was obtained in the planting of May 11; high infections, however, occurred in the plantings of April 6 and 20. The percentage of infection in the planting of March 29 was somewhat less, and there was a decided decrease in that of May 31. The average temperatures during the periods of germination were 46°, 60.3°, 55.1°, 68.5°, and 73.5°F. The rainfall during the corresponding periods was 0.81 in., 0.27 in., trace, 0.05 in., and 0.84 in.

In addition to these two susceptible varieties, three very resistant ones also were grown in this experiment: *Avena brevis* Roth (S. N. 289), *A. sativa* L. var. *Black Mesdag* (S. N. 70), and *A. sterilis* L. var. *Fulghum* (S. N. 129). Six plants of *A. brevis*, out of a total of 1268, were infected by *Ustilago levis*. These were found in the seedings of March 29, April 6, and May 31. Two plants of *Fulghum*, one in the seeding of March 29 and the other in the seeding of May 11, out of a total of 686, also were infected by *Ustilago levis*. These two varieties gave entirely negative results with *Ustilago avenae*, and *Black Mesdag* gave negative results with both smuts.

A large number of observations have been made on the relation between

date of planting and the prevalence of bunt of wheat, but different observers have recorded quite divergent results. These data have been discussed by the senior author (37).

*Influence of Environmental Factors on Smut Infection.* Brefeld (5) was the first to attempt to determine the influence of temperature on the infection of oats by smut. He germinated at 7° C. oats inoculated with conidia and obtained 40 to 46 percent infection. On the other hand, oats inoculated and germinated at 15° gave only 27 to 30 percent infection.

Herzberg (13) stated that the cardinal points for the germination of the spores of *Ustilago avenae* are: minimum 5°–11°, optimum 22°–30°, and maximum 30°–35° C. He also found that the cardinal points for mycelial growth are: minimum, slightly under 6°, optimum 18°–26°, and maximum 30°–34° C. Von Tubeuf (40) in a series of pot experiments obtained 1 percent infection of the seedlings germinated at temperatures of 1°–9° C., 12–20 percent infection at 10°–20°, and 22–24 percent infection at 20°–21°. He failed to observe germination of oat smut spores at 2½°–8° C. Hecke (12) recorded the following cardinal temperatures for oats: minimum 4°–5°, optimum 25°, maximum 30° C.; and for *Ustilago avenae*: minimum, 5°–11°, optimum 22°–30°, maximum 30°–35° C.

Bartholomew and Jones (3) have studied the influence of temperature and moisture on the infection of *Avena nuda* L. var. *inermis* by *Ustilago avenae*. They found that at temperatures between 15° and 23° C., combined with low soil moisture, 100 percent of the plants were infected. The percentage of infection was decreased at lower as well as at higher temperatures. They found, further, that plants in a soil moisture of 67 percent consistently gave lower percentages of infection than in a soil moisture of 36 percent, and soil moisture of 80 percent gave still lower percentages. The cardinal temperatures for *Avena nuda* are recorded as: minimum 7°, optimum 19°, and maximum 33° C. They obtained some evidence of germination in *Avena nuda* at temperatures of 3–5° C., but no subsequent growth occurred. The exact maximum temperature also was not determined. Jones (20) found that the cardinal temperatures for the germination of the spores of *Ustilago avenae* are: minimum 4–5°, optimum 15–28°, and maximum 31–34° C. The temperatures for sporidial production are the same except that the maximum is 30° C.

Kulkarni (27, 28) has suggested that soil temperature is an important factor in the infection of sorghums by *Sphacelotheca sorghi*. He obtained 54.6 percent infection in plants germinated at a temperature of 25° C., but no infection in plants germinated at 40°. In field experiments no infection occurred at Jacobabad where the temperature at the time of seeding was 30°–40°. On the other hand, 65 percent infection occurred at Poona where the temperature at the corresponding period was 25°. Kulkarni also found that sorghum germinated most rapidly at 37°; on the other hand, very few spores of *Sphacelotheca sorghi* germinated at this temperature. The



temperature for the maximum germination of spores was  $20^{\circ}$ – $30^{\circ}$ , and at this temperature the sorghum required one to two days longer to germinate than at  $37^{\circ}$ .

The senior author found in his experiments at Columbia, Missouri, on the varietal resistance of sorghums to *Sphacelotheca sorghi* during the years 1915–1918 inclusive, that the percentages of infection obtained in 1918 were very much lower than during the other three seasons. The temperature in 1918 was much higher, averaging  $74\frac{1}{2}^{\circ}$  F. for the seven days preceding planting and  $81^{\circ}$  F. for the seven days following planting. In the other three seasons the corresponding temperatures were  $67^{\circ}$ – $70^{\circ}$  F. for the first period and  $69\frac{1}{2}^{\circ}$ – $70^{\circ}$  F. for the second period. The season 1918 also was unusually dry, both during the planting season and for some time previous.

In the light of our present experiments, one might expect that high infections would have been obtained in 1918 in the dry soil instead of low infections, since low soil moistures at  $20^{\circ}$  C. have consistently been the most favorable for high infection. It is possible, however, that at higher temperatures a low soil moisture would not be so favorable. This is clearly indicated by our results with the oat smuts.

Rose (39) germinated barley at  $4^{\circ}$ – $9^{\circ}$  C. but obtained no infection. On the other hand, seed germinated at  $22\frac{1}{2}^{\circ}$ – $27^{\circ}$  gave 13.5 percent infection. He also planted barley on March 30 but obtained only 0.7 percent infection, while a planting on May 23 gave  $2\frac{1}{2}$  percent. Less smut developed in barley grown in clay soil than in that grown in sandy, humus, or loamy soil. Unfortunately, Rose did not clearly distinguish between the loose and covered smuts of barley, hence his results are of uncertain application.

The junior author (9) has made extensive studies on the influence of environal factors on infection of barley by *Ustilago hordei* (Pers.) K. & S. His seeds were germinated in soils of two moistures (40 and 50 percent) and four pH reactions at six different temperatures from  $5^{\circ}$  to  $30^{\circ}$  C. The average of the two moistures and four pH values gave 6.7 percent infection at  $5^{\circ}$  C.; 60.6 percent infection at  $10^{\circ}$ ; 61.4 percent at  $15^{\circ}$ ; 53.9 percent at  $20^{\circ}$ ; 35.7 percent at  $25^{\circ}$ ; and 3.7 percent at  $30^{\circ}$ . High infections occurred over a wide range of temperature,  $10^{\circ}$ – $25^{\circ}$  C. In general, he found higher percentages of infection at all these temperatures in a soil moisture of 50 percent than in one of 40 percent, provided the soil was acid in reaction.

Several investigators have endeavored to determine the relation of moisture, temperature, and other soil factors to the infection of wheat by bunt. Von Tubeuf (40) obtained 55.3 percent infection in wheat planted in sandy soil and watered, as compared with 29.3 percent infection of plants in similar soil but not watered. Volkart (42) accounts for the greater infection of wheat in his early planting as compared with later on the basis of a soil moisture more favorable for the rapid germination of the wheat.



Malkoff (29) found different amounts of smut in several varieties of wheat in two successive years. His analysis of the meteorological conditions led him to conclude that the amount of moisture in the soil during the germination of the seed had a very marked effect upon the extent of the infection. Appl (2) concluded that the small differences in the temperature ranges were not sufficient to explain his results. From his examination of the records on the amount of precipitation during the period of germination he concluded that soil moisture is of greater importance than temperature for successful infection. Hungerford (18) also concluded that moisture is particularly important in the infection of wheat by bunt in those regions where soil infestation occurs.

Hecke (12) attempted to correlate the amount of bunt in spring wheat with the temperature conditions. His highest percentages of infection were obtained in the early sowings, although some rather marked exceptions occurred. In general, Hecke found that with low temperatures there was a higher percentage of infection. Munerati (31) obtained 1.4–12 percent infection in four varieties of wheat germinated at 18°–20° C. These same varieties, germinated at 7°–8°, gave 92–100 percent infection. More recently, Munerati (32) has reported some additional results on the temperature relations of the infection of wheat. He obtained the following results with two different varieties: Seed germinated at 22°–25° gave 0–1.4 percent infection; germinated at 10°–12° gave 13.2–39.3 percent; germinated at 2°–4° for forty days gave 0–1.9 percent; germinated at 2°–4° for twenty days, then at 22°–25°, gave no infection; germinated at 2°–4° for twenty days, then at 10°–12° for seven days, and finally at 22°–25°, gave 19.7–24.4 percent. Heuser (14) secured 1.8–6 percent infection in three varieties germinated at 16°–22°, and 67.1–94.7 percent in the same varieties germinated at 6°–10°.

Prevost (33) appears to have been the first investigator to study the influence of temperature on the germination of bunt spores. He found that the number of days required for their germination at 17°–18° C. was 2½–3 days; at 14°–15°, 3–4 days; at 11°–12°, 5–6 days; at 7°–8°, 9–10 days, and at 4°–5°, 11–12 days. Von Tubeuf (40) obtained no germination of bunt spores at 3°–8° C. Five days were required for germination at 11½°–16½°. Volkart (42) found that wheat germinated much more quickly at 5.3° C. than bunt spores. There was very little difference in the time of germination at temperatures of 12.3°–19.1°. Bunt spores did not germinate at temperatures of 30.7° or above. Hecke (12) found the cardinal temperatures for the germination of wheat to be: minimum 3°–4½°, optimum 25°, and maximum 30°–32½° C. The corresponding temperatures for the germination of bunt spores were: minimum 5°, optimum 16°–18°, and maximum 25° C.

Other conditions have been emphasized as playing a part in the infection of hosts by the cereal smuts. Clinton (7), McAlpine (30), and Bartholomew and Jones (3) obtained some evidence that presoaking the seed before

inoculation or inoculating wet seed favors infection. Jensen (19) called attention to the possible influence of the hulls on infection of oats and barley. Clinton (7) also obtained higher infection in oats when the hulls were removed as compared with the same variety with the hulls on. He further obtained some data indicating that an increase in the depth of planting results in slightly greater infection.

McAlpine (30) found that the number of spores per grain has a marked effect in increasing the percentage of infection. Heald (11) found 0.5 gram of powdered bunt spores well distributed to 100 grams of seed necessary to produce the maximum infection of wheat. A spore load of 36,000-156,000 spores was required to produce the maximum percentage of smut, and the amount of smut in the crop up to a certain point increased with the spore load. Munerati (31) also has considered the influence of spore load and has emphasized the fact that the location of the spores on the seed is of much greater importance than the total number. He obtained very slight infection of wheat by bunt when the spores were restricted to the apical zone of the grain. In contrast, quite high percentages of infection occurred when the spores were placed upon the embryonal zone. Temperature, as already noted, markedly influenced his results.

*Influence of Rate of Germination of Seed on Smut Infection.* Many investigators have attempted to explain the variation in infection under diverse conditions by differences in the rate of germination and subsequent growth of the seedlings of the host. Brefeld (4) clearly recognized that seedlings may differ greatly in their rate of development and that their susceptibility to smuts may depend upon this difference. Hastened or retarded development during germination may be the determining factor in infection. He emphasized the probable influence of temperature. Since temperature, however, may act upon the growth of the seedlings as well as upon the fungous mycelium in their tissues, he endeavored to determine the effect of temperature on host and parasite. He obtained a slightly greater percentage of infection of seedlings germinated at 15° C. as compared with higher temperatures, and he concluded that the increased temperature hastened the growth of the seedlings more than it did that of the fungous mycelium and thus prevented the development of the latter in the floral organs of the host.

The notion that there is a definite relation between the occurrence of smut in cereals and the rate of development of the germinating seedlings has been generally accepted. Kellerman and Swingle (22) suggested that the greater amount of smut in late-planted fall wheat is due to the fact that the wheat grows more slowly than that planted earlier and consequently is exposed to a greater chance of infection. This same idea has been variously expressed by von Tubeuf (40), Appel and Gassner (1), Hiltner (16), McAlpine (30), Malkoff (29), Volkart (42), Heuser (14), and Munerati (31).

Malkoff (29) accounted for the greater susceptibility of Durum wheats to

*Tilletia laevis* on the basis of their less rapid germination. In his own experiments, soil moisture appeared to be more effective in influencing the rate of germination than temperature.

Von Tubeuf (40) suggested that there is an internal predisposition in susceptible varieties. He has also suggested that many varieties of cereals are distinguished by the time required for their germination, for the lengthening and hardening of the tissues, or for the attainment of a condition in which infection is no longer possible. At low temperatures wheat germinates somewhat slowly, and the result is that before the wheat has passed the susceptible stage, the smut spores also have germinated and infection may occur. On the other hand, at higher temperatures the wheat germinates more rapidly and quickly passes through the susceptible stage before the smut spores have germinated. In this way he accounts for the increase in the amount of bunt in wheat when the seed is germinated at fairly low temperatures. The conditions in oats, however, are quite different, since moderate temperatures influence favorably both the smut spores and the germination of the seed.

Appel and Gassner (1) have endeavored to show that susceptible varieties germinate and pass through the later stages of development more slowly than resistant varieties. McAlpine (30) has reported similar results. Hiltner (16) has emphasized the fact that Fichtelgebirgs oats, which are highly susceptible to oat smut, may germinate as rapidly as the resistant Ligowo but that in the later stages of seedling development there is a marked retardation, and on this fact is based his explanation of the greater susceptibility of Fichtelgebirgs oats. Burk (6) has reported differences in the germination and subsequent development of wheat varieties, but has given no data correlating these differences with susceptibility to bunt.

Von Kirchner (24, 25, 26), however, was unable to find any correlation between the rate of germination of wheats and their susceptibility or resistance to bunt, and he concluded that the variation in the susceptibility of different wheats is an hereditary peculiarity of the variety and depends upon differences in the internal composition of the cells. He stated, however, that within the variety the amount of infection may be influenced by external factors, as temperature, moisture, nutrition, etc. Darnell-Smith (8) observed no differences in the germination of wheats susceptible and resistant to bunt.

Hecke (12) has emphasized a three-fold influence of temperature: first, the direct effect of temperature upon the germination of the smut spores and of the seed; second, the influence of temperature upon the duration of the susceptible stage of the host plant; and third, the influence of temperature in its effect upon the possibility of the fungus reaching the growing point of the host.

It is clear from the data which we have presented that there is a definite interdependent relation between various factors—moisture, temperature,

and soil reaction—and the infection of oats by *Ustilago levis* and of sorghums by *Sphacelotheca sorghi* and *S. cruenta*. It is also clear from our results that the ranges for temperature, moisture, and soil reaction are fairly wide.

In our studies we kept records of the germination of the seeds in the different experiments. As already stated, the seeds were planted at a uniform depth of one inch and records were taken of the number of days required for the seedlings to appear above the surface of the sand. In each experiment 150 seeds of the varieties of sorghum and 60 seeds, except in two or three cases, of the oats were planted. The oats as a rule gave excellent germination, but the sorghums gave comparatively poor results. In some of the experiments a few additional seedlings may have emerged after the final date of observation. The percentages of infection for the sorghums are taken from our previous paper (38) and for the oats from

TABLE 6. Influence of Temperature on Germination of Sorghums and Infection by *Sphacelotheca sorghi*, 1923; Sand, 30 percent Moisture; pH, 7.2

Variety	Seed No.	15° C.											Infection %
		Germination and Emergence											
		Days 9	Days 10	Days 11	Days 12	Days 13	Days 14	Days 15	Days 16	Days 17	Days 18		
Darso .....	225	0	13	34	55	61	67	68	72	75	76	0	
Kafir, Blackhull. ....	223	0	0	7	19	28	36	47	55	60	63	59.5	
Kaoliang, Valley. ....	192	3	44	84	111	112	119	121	124	124	128	58.6	
Sorgo, Red Amber. ....	232	0	4	12	48	63	69	73	79	87	88	23.1	

Variety	Seed No.	20° C.					25° C.					Infection %
		Germination and Emergence				Infection %	Germination and Emergence				Infection %	
		Days 4	Days 5	Days 6	Days 7		Days 3	Days 4	Days 5	Days 6		
Darso .....	225	22	73	86	89	0	27	93	94	—	0	
Kafir, Blackhull. ....	223	5	45	73	88	50.0	4	86	97	III	53.2	
Kaoliang, Valley. ....	192	88	123	—	—	77.4	75	130	131	—	63.4	
Sorgo, Red Amber. ....	232	23	86	95	105	21.7	29	109	116	—	37.8	

Variety	Seed No.	30° C.					35° C.					Infection %
		Germination and Emergence			Infection %	Germination and Emergence		Infection %				
		Days 2	Days 3	Days 4		Days 2	Days 3					
Darso .....	225	0	82	95	0	86	100	0				
Kafir, Blackhull. ....	223	0	79	109	35.0	46	102	1.6				
Kaoliang, Valley. ....	192	34	115	—	42.2	61	75	14.5				
Sorgo, Red Amber. ....	232	2	104	114	14.5	66	113	2.2				

tables 1 and 2. These percentages are based on the total number of plants which grew to maturity. Table 6 gives the results obtained on the influence of temperature on germination of sorghums and on infection by *Sphacelotheca sorghi*.

It will be noted that Valley Kaoliang invariably germinated most rapidly. The first seedlings of this variety appeared above the soil earlier than those of any other variety. In every case, with one exception, the percentage of infection of this variety was higher than that of any other. The only exception occurred at 15° C., at which temperature Blackhull Kafir gave a slightly higher percentage of infection.

Blackhull Kafir was the slowest variety to germinate. This variety ranked in nearly every case next to Valley Kaoliang in susceptibility. Darso germinated on an average somewhat more rapidly than Red Amber Sorgo. In every case, however, it gave negative results with *Sphacelotheca sorghi*, while Red Amber Sorgo was moderately infected, depending upon the temperature.

Records were also kept of the germination of *Avena nuda* L. var. *inermis* and *A. sativa* L. var. Victor grown under different temperatures and moisture conditions. The results are given in table 7. At 5° C., *Avena nuda* germinated somewhat more slowly than Victor. On the 26th day seedlings began to appear above the surface of the sand in both varieties, but they were more numerous in Victor than in *A. nuda*. Additional seedlings continued to appear on the following days, and by the end of the 30th day practically all the seedlings of Victor had emerged while about two-thirds of those of *Avena nuda* were up. At this temperature, *A. nuda* was somewhat slower in germination but was consistently more severely infected.

At 10° C., about 50 percent of the seedlings of *Avena nuda* were up on the tenth day, whereas of Victor only two had appeared. On the 12th day all

TABLE 7. Influence of Temperature and Moisture on Germination of Oats and Infection by *Ustilago levis* (K. and S.) Magn.

5° C.

% Moisture	<i>Avena nuda</i> (S. N. 30)					<i>Avena sativa</i> var. Victor (S. N. 126)				
	Germination and Emergence				Infection %	Germination and Emergence				Infection %
	Days 26	Days 28	Days 29	Days 30		Days 26	Days 28	Days 29	Days 30	
15	0	9	18	24	75.0	0	3	7	11	0
20	1	4	7	9	51.5	17	42	50	60	19.2
25	13	27	31	34	30.5	18	47	54	61	6.5
30	5	20	24	32	38.4	14	41	46	57	0
35	8	27	33	36	32.5	12	43	49	55	0
40	14	26	30	34	19.1	25	50	57	59	0
50	13	35	41	44	18.5	30	55	61	63	2.7
60	10	27	35	38	11.7	13	41	51	58	0

(Table 7.—Continued)

10° C.

% Moisture	<i>Avena nuda</i> (S. N. 30)				<i>Avena sativa</i> var. Victor (S. N. 126)				
	Germination and Emergence			Infection %	Germination and Emergence				Infection %
	Days 10	Days 11	Days 12		Days 10	Days 11	Days 12	Days 13	
15	22	50	56	74.0	0	10	33	45	43.1
20	9	43	50	67.8	0	8	34	48	47.0
25	15	42	51	56.1	0	23	41	52	28.3
30	22	51	58	50.8	0	22	37	45	12.5
35	16	46	53	35.2	0	13	42	54	29.0
40	18	46	53	42.0	1	15	29	34	17.1
50	20	43	50	29.1	1	19	44	55	10.0
60	18	47	53	34.4	0	7	39	47	19.2

15° C.

% Moisture	<i>Avena nuda</i> (S. N. 30)			<i>Avena sativa</i> var. Victor (S. N. 126)			
	Germination and Emergence		Infection %	Germination and Emergence			Infection %
	Days 6	Days 7		Days 6	Days 7	Days 8	
15	7	43	92.6	0	25	53	87.5
20	18	55	78.5	0	21	59	48.0
25	10	50	66.1	0	28	59	22.6
30	25	50	78.8	0	37	62	33.8
35	43	57	54.2	3	38	47	26.5
40	46	56	73.3	6	41	51	36.9
50	34	53	62.2	1	35	42	25.5
60	29	52	65.4	2	44	54	21.1

20° C.

% Moisture	Avena nuda (S. N. 30)				Avena sativa var. Victor (S. N. 126)			
	Germination and Emergence			Infection %	Germination and Emergence			Infection %
	Days 4	Days 5	Days 6		Days 4	Days 5	Days 6	
15	2	11	18	95.2	0	0	0	66.6
20	8	30	38	82.9	0	1	4	93.7
25	14	53	—	88.8	0	19	25	85.0
30	15	56	—	74.5	0	34	45	58.6
35	20	56	—	58.9	0	46	56	39.2
40	31	55	—	67.8	0	42	52	46.6
50	37	55	—	70.5	0	43	55	40.0
60	32	58	—	42.1	0	48	57	48.0



the seedlings of *Avena nuda* were up, while the germination of Victor was completed on the 13th day. At this temperature *A. nuda* consistently germinated more rapidly than Victor. However, it was again much more severely infected.

At 15° C., seedlings of *A. nuda* had emerged on the 6th day and germination was completed on the 7th. On the other hand, only a few seedlings of Victor appeared on the 6th day, somewhat more than 50 percent on the 7th, and germination was completed on the 8th and 9th days. Again *Avena nuda* germinated more rapidly and was also more severely infected. At 20° C., the seedlings of *Avena nuda* were appearing on the 4th day, and on the 5th day practically all had emerged. With Victor, on the other hand, no seedlings were observed on the 4th day, but by the 5th most of them had emerged, the last of them coming up on the 6th or later days. At this temperature *Avena nuda* germinated most rapidly and was the most severely infected in practically every moisture.

At all the different temperatures, the germination of both Victor and *A. nuda* was slightly retarded in the lower moistures. Irregularities, however, occur, so that a general statement can not be definitely made. It is also true that the higher percentages of infection were generally obtained in the lowest moistures.

It is evident, however, that the greater susceptibility of *Avena nuda* as compared with Victor to *Ustilago levis*, can not be explained by differences in the rate of germination of the seeds. It is also evident that the difference in susceptibility of the sorghums does not depend upon differences in their rate of germination. Valley Kaoliang in almost all cases appeared to be the most susceptible and also the most rapid in germination. Blackhull Kafir regularly gave very high percentages of infection, but it had the slowest rate of germination. The seedlings of Darso germinated at a rate somewhat intermediate between those of Valley Kaoliang and Blackhull Kafir, but this variety was not susceptible to *Sphacelotheca sorghi*.

It is evident, therefore, that there are real hereditary differences in the susceptibility and resistance of these different varieties. It is also evident that environmental conditions, as temperature, moisture, and soil reaction, may greatly influence the amount of infection, but this does not seem to be in any way connected with a change in rate of germination.

The further question may be raised as to why Valley Kaoliang and Blackhull Kafir never gave 100 percent infection with *S. sorghi*. These varieties are evidently very susceptible to their respective smuts, but 100 percent infections were not obtained in any case. It may be that the explanation for the failure of infection in a considerable percentage of the plants of such varieties rests upon a difference in the rate of growth of individual seedlings. It is evident from the records that the individual seedlings varied considerably in their germination.

There is some evidence that factors which influence the later development



of the plant may have a bearing upon the appearance of smut in the floral organs. Heuser (14) has attempted to show that variations in nutrition influence the percentage of bunt in wheat. He found considerably less bunt in wheat fertilized with nitrogen than in wheat unfertilized. Unfortunately, his data are based very largely upon head counts and not upon the number of infected plants, and consequently do not throw much light upon this particular problem. It is possible that many plants may be penetrated by the fungus but that the mycelium fails to develop and reach the floral organs.

Hiltner and Lang (17) have made rather extensive studies on the influence of fertilization, especially with compounds of nitrogen, upon the severity of smut in cereals. They found a marked decrease in the amount of smut when a considerable quantity of nitrogen was used in fertilizing the soil. Different amounts of the substances used seemed to have marked effects upon the development of the smut.

Finally there may be noted the remarkable correspondence in the behavior of *Ustilago avenae* and *U. levis*. Bartholomew and Jones (3) studied the former species in relation to *Avena nuda* and found that infection is possible over a wide range of temperature and moisture. Low soil moistures favor infection as compared with high soil moistures. They did not succeed in inducing *Avena nuda* to germinate at as low a temperature as in our experiments. This was probably due to the fact that they failed to keep the seeds a sufficient length of time.

There is also a very great similarity in the behavior of *Sphacelotheca sorghi* and *S. cruenta*. Both species are able to infect susceptible varieties over wide ranges of temperature, moisture, and soil reaction, and they seem to be influenced in much the same fashion by these different factors. Our results also indicate a wider range for infection by *S. sorghi* than those reported by Kulkarni (28), although high temperatures are unfavorable for severe infections.

#### SUMMARY

1. The influence of temperature, moisture, and soil reaction upon the infection of two varieties of oats by *Ustilago levis* (K. & S.) Magn. and of several varieties of sorghum by *Sphacelotheca sorghi* (Link) Clinton and *S. cruenta* (Kühn) Potter have been studied.

2. *Avena nuda* L. var. *inermis* (S. N. 30) and *A. sativa* L. var. Victor (S. N. 126) have been grown at six different temperatures from 5° to 30° C. and at eight different moistures. *Avena nuda* became infected at all the temperatures. If the results for the different moistures are added together, the percentages of infection, beginning with 5° C., are 32.1, 49.1, 71.2, 70.0, 83.2, and 18.2. The highest infections occurred at 25° C. High infections, however, were also obtained at 15° and 20° C. Victor was also infected at all the temperatures, but consistently gave lower results than *Avena nuda* except at the highest temperature, 30° C. Averaging the results obtained at all moistures, the results for Victor are 3.3, 25.9, 37.4, 57.3, 63.8 and 20.5

percent. Again the highest infections occurred at 25° with severe infections at both 15° and 20° C. *Avena nuda* gave higher infections at 5° C. and lower at 30° C. than Victor. *Avena nuda* gave infections over a range of moistures from 15 to 80 percent. The highest percentages of infection were obtained in the lower moistures. Similar results were obtained with Victor in moistures of 15 to 60 percent. Infections of both *Avena nuda* and Victor were obtained over a rather wide range of soil reactions. It was found, however, that soil near the neutral point, or only slightly acid, was most favorable for high infections.

3. A number of susceptible varieties of sorghums were grown at temperatures of 12° to 37.5° C. and at soil moistures of 10 to 80 percent. Infection occurred in susceptible varieties over a wide range of temperature. In every case infections were secured at 15° and in nearly all cases at 35° C. Infections also occurred over a wide range of moistures, with particularly high infections in the lower soil moistures. Infections also occurred over a rather wide range of soil reactions, slightly acid soils being the most favorable for high infections.

4. The results with the two species of sorghum smuts, *Sphacelotheca sorghi* and *S. cruenta*, were very similar. They responded in similar fashion to temperature, moisture, and soil reaction in their infection of susceptible varieties.

5. Several resistant varieties of sorghums were grown at a number of different temperatures, and Darso, resistant to *Sphacelotheca sorghi* but susceptible to *S. cruenta*, was grown at a number of different moistures as well. Under all these conditions the resistant varieties consistently maintained their freedom from infection.

6. Soil moisture, soil temperature, and soil reaction are interdependent factors. Their interaction determines whether infection will take place and also the severity of the attack. Any one of these factors may be a limiting one in the prevention of infection. The term "optimum temperature for infection," for example, is without significance unless due regard is paid to other possible influencing factors.

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## THE RELATION OF ENVIRONMENT TO DISEASE IN PLANTS<sup>1</sup>

L. R. JONES

In plant pathological studies I believe the time has come when we should put relatively less stress upon the parasite as an independent organism and relatively more on the disease. As the first step in this, I would urge increased attention to the relation of environment to disease-inception and development. Correlated with this should be more work upon the physiology of parasitism with critical inquiry into the influence of environment upon fungous life cycle and infection.

For a generation past, pathologists have concentrated attention upon the parasite. Has not the germ theory of disease in its simplest conception—"germs" synonymous with disease—so filled the mind and satisfied the imagination of public and practitioner that it has been natural for even the investigator, who should see more deeply into his problem, to be influenced by the popular measure of his responsibilities? Has it not often been temptingly easier to find and name a new fungus than to probe patiently into the behavior of one long known? When the potatoes show scab or scurf and the grower asks "why," have we not been too prone to ease our mind, and perhaps mislead his, by pronouncing some of our modern mystic words "Actinomyces," "Rhizoctonia"? When we so frequently must face the fact, with these and many other diseases, that, comparing field with field and season with season, the loss following like initial infection may vary widely—perhaps even from nothing to total—then we must agree that naming the parasite is only a first step in explaining disease-occurrence.

In thus urging increased attention to environment as influencing disease, I may lead you to a wrong inference. I am not here to urge that a majority of our workers make specific environmental problems matters of major concern. When started, these require persistent application which not all can give, and the methods are not simple and direct. Probably the chief responsibility should be left with a relatively limited number of men in a still more limited number of institutions. With increasing definiteness of purpose let us follow our biological instincts for "division of labor," especi-

<sup>1</sup> Invitation paper read at the joint meeting of Section G, American Association for the Advancement of Science, the Botanical Society of America, and the American Phytopathological Society at Cincinnati, December 28, 1923.

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ally where correlated "differentiation of structure" is required as exemplified by special and expensive apparatus.

I am here, however, to stress the plea that from now on every one working with a specific disease, whether upon cause, occurrence, or control, may be keenly alert to the significance of environment as a factor in his problems. By critical attention to this, the discriminating observations of the many workers, in different areas and with varying conditions, will add rapidly to our store of general knowledge regarding these matters. From these widespread observations will come many useful data for correlation, and also the clearer definition of specific problems to which the intensive worker may apply himself. Moreover, it is only through such widespread interest that critical review will be insured for such pronouncements as come from those making the more specialized investigations.

Work with environmental problems is difficult and exacting, while the results lack satisfying definiteness. We are not ourselves virtuously fond of such tasks, and we are ready to confess that this work was not seriously undertaken at Wisconsin until it was forced upon us.<sup>2</sup> A decade ago we seemed well along with our simple and satisfyingly limited undertaking of locally controlling the cabbage yellows disease caused by a soil fungus, a vascular *Fusarium*. Our disease-resisting strains seemed at first fully to meet the needs of the case. Soon, however, we were disturbed by finding a distressingly wide seasonal variation in the results with even the most resistant types. Almost perfect freedom from disease obtained one year, followed by 25 percent or more the next! What did it mean? The severity of the disease, and conversely the expression of disease resistance, were influenced by environment. Disease resistance is in general only relative, and therefore may be expected to "break down" in some degree under certain conditions. Before long requests began to come for the resistant Wisconsin seed to be sent to other states. Of course, we could not with equanimity contemplate sending our pet cabbage seed even to trustworthy friends in Iowa and in Virginia, when we knew not how badly their climatic conditions might upset our Wisconsin-made predictions. Clearly it was necessary to continue the search until we had adequate understanding of the relation of varying environment to the occurrence of this cabbage yellows and to the performance of our Wisconsin disease-resisting strains.

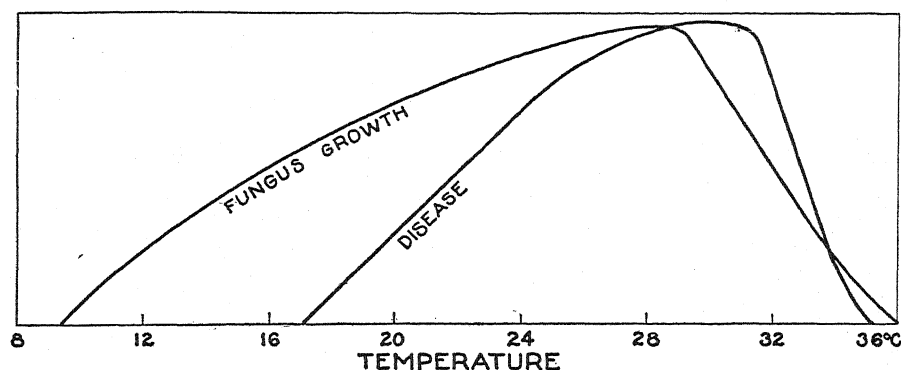
Again this problem arose, and in almost exactly the same form, as our associate James Johnson advanced his work with the *Thielavia* root rot of tobacco and its control through disease-resisting strains.<sup>3</sup> These things necessitated the critical studies with soil temperature, earlier begun by

<sup>2</sup> For citations as to methods and development of these Wisconsin investigations reference may be made to Jones, L. R. Experimental work on the relation of soil temperature to disease in plants. *Trans. Wis. Acad. Sci. Arts Lett.* 20: 433-459. 1921.

<sup>3</sup> Johnson, J., and Hartman, R. E. Influence of soil environment on the rootrot of tobacco. *Jour. Agric. Res.* 17: 41-86. 1919.



Gilman<sup>4</sup> and Tisdale<sup>5</sup> and later advanced through the development of the Wisconsin soil-temperature tanks. The fact was soon experimentally demonstrated that certain soil-borne diseases, including cabbage yellows, flax wilt, tomato wilt, and tobacco root rot, are clearly conditioned in their



TEXT FIG. 1. Tomato wilt. Graphic comparison of the influence of incubator temperatures (degrees Centigrade) upon the growth of the fungus *Fusarium lycopersici* with that of the corresponding soil temperatures upon the development of the tomato-wilt disease caused by it. Note the close parallelism of the two curves, the optimum in each alike being at about 28° C.

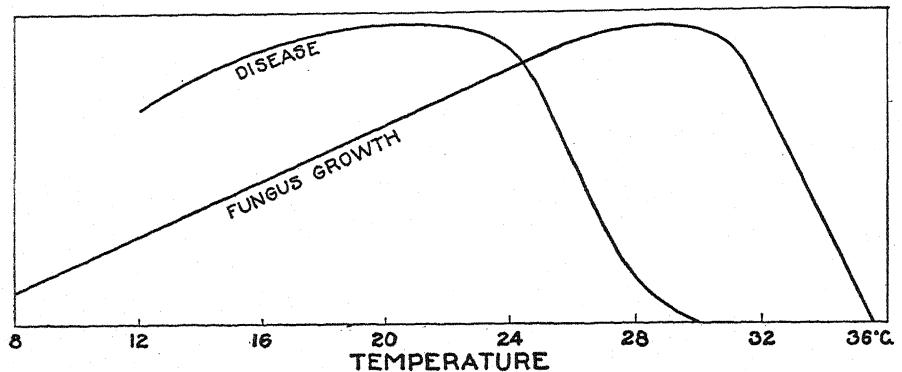
occurrence upon definite environmental factors. In each case, even in the "sickest soil," the disease was experimentally developed in destructiveness ranging consistently from 100 percent of the crop to zero, with change of only one factor, soil temperature, and this well within the temperature range reasonably congenial to the respective host. Most suggestive and stimulating of all, however, was the fact that these diseases do not behave alike. Cabbage yellows, flax and tomato wilt are favored by high soil temperature (text fig. 1), tobacco root rot by low soil temperature (text fig. 2).

With these suggestive results before us, the experimental tanks available, and graduate students eager for problems, it was a matter of course that we should at Wisconsin try to determine the influence of soil temperature upon other soil diseases. The vascular *Fusarium* diseases worked with have all been favored by high temperature and have a sharply limited low-temperature range. In general, the temperature growth curve for the *Fusarium* in pure culture corresponds closely with that for the development of the disease, as illustrated in text figure 1. This suggests that with these examples the direct influence of temperature upon the vegetative activities of the parasite may be the dominant influence.

<sup>4</sup> Gilman, J. C. Cabbage yellows and the relation of temperature to its occurrence. *Ann. Mo. Bot. Gard.* 3: 25-84. 1916.

<sup>5</sup> Tisdale, W. H. Relation of temperature to the growth and infecting power of *Fusarium lini*. *Phytopath.* 7: 356-360. 1917.

But with numerous other cases like the potato *Rhizoctonia*,<sup>6</sup> just as with the *Thielavia* root rot of tobacco, these two curves are antagonistic, as illustrated in text figure 2. *Thielavia* and *Rhizoctonia* in pure culture are



TEXT FIG. 2. Tobacco root rot. Graphic comparison of the influence of incubator temperatures (degrees Centigrade) upon the growth of the fungus *Thielavia basicola* with that of the corresponding soil temperatures upon the development of the tobacco root rot disease caused by it. Comparison with text figure 1 will show that the influence of temperature on fungous growth is essentially the same in the two examples, with growth optima alike at about 28° C. There is, however, a marked difference in the disease curves. The tobacco root rot develops most seriously at a temperature distinctly below the optimum for fungous growth.

alike stimulated to more rapid growth with rising temperature, having an optimum, like the *Fusariums*, at about 28° C., yet in each case respectively this same rise of temperature which stimulates the fungus soon checks the disease, until at the optimum for fungous growth the disease is wholly inhibited.

What is the reason? The first question in these studies of environment is, what is the effect of environment, and of each factor of environment, on the development of a specific disease? The second and more difficult question which forces itself immediately after the first is, why this effect in each particular case? In how far may it be due primarily to influence on parasite, in how far primarily to influence on host, and in how far to a combination of these?

Quite specific evidence bearing on this has come from studies at Wisconsin in which Walker and Dickson have been the leaders, dealing with onion smut on the one hand and the *Fusarium* seedling blights of cereals on the other. It was found that onion smut,<sup>7</sup> like the *Thielavia* root rot of tobacco, is a low-temperature disease, decreasing with rising temperature

<sup>6</sup> Richards, B. L. Pathogenicity of *Corticium vagum* on the potato as affected by soil temperature. Jour. Agric. Res. 21: 459-482. 1921.

<sup>7</sup> Walker, J. C., and Jones, L. R. Relation of soil temperature and other factors to onion smut infection. Jour. Agric. Res. 22: 235-261. 1921.

and eliminated entirely at about 28° C. Recent inquiries into the cause, of which one of our younger students, Wellman,<sup>8</sup> has carried out the details, show that with rising temperature the smut-spore germination is inhibited, and that this germination ceases at 28° C. In other words, the temperature-spore-germination curve here almost exactly parallels the temperature-disease-development curve. Since we interpret this disease as primarily arising from infection resulting from soil-borne smut spores, it would seem that the direct influence of soil temperature upon spore-germination, either stimulating or inhibitory, might offer sufficient answer to the second question formulated above. On the other hand, it seems probable that there is also some influence of temperature upon the character of the onion host tissue, making it more resistant or more susceptible to penetration, which may have a bearing upon the matter. This, however, yet awaits further study.

In Monteith's recent work at Wisconsin upon relation of environment to club root of cabbage,<sup>9</sup> he judges that of the two factors he worked with one may operate primarily upon the parasite, the other upon the host. Of course it has long been recognized that soil reaction strongly influences club root, and it has been inferred that the influence is primarily on the parasite. With this Monteith did not work. He did, however, show that soil moisture has a decided influence which may possibly even dominate that of soil reaction. The disease increases with increasing moisture content, even up to soil-saturation, and this disease-increase, at least at the lower moisture stages, is apparently due to the need of water for the spore-germination and possibly for migration and infection. With decreasing moisture, the disease may be eliminated at a point at which the cabbage still secures sufficient water for normal head-development. The amount of clubbing also increases with rising temperature, but here the progress seems directly correlated with amount of root growth; in other words, the influence of temperature seems primarily exerted on the host.

A still more significant and complete analysis of the relation of environment to the host, as compared with the parasite, has come from the work of Dickson and his associates<sup>10</sup> upon the seedling blights of corn and wheat. Here the one organism, the Fusarium stage of *Gibberella saubinetii*, is capable of parasitizing the seedlings of the two hosts, causing a like seedling-blight disease. Fortunately, these investigations dealt with two hosts

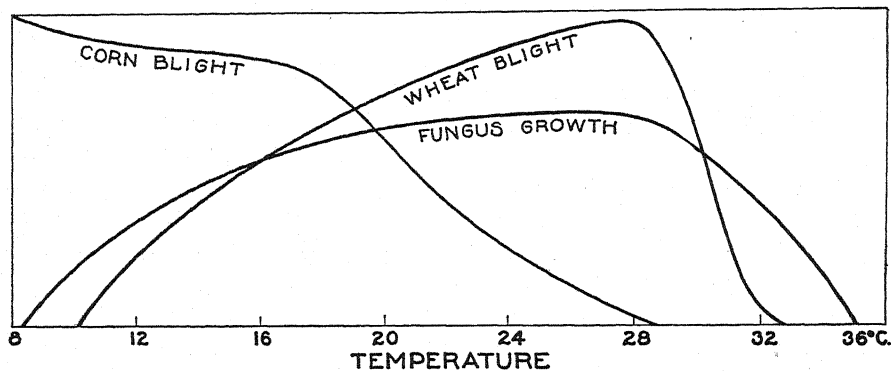
<sup>8</sup> Walker, J. C., and Wellman, F. L. Temperature relations of *Urocystis cepulae* (Frost) (Abstr.). *Phytopath.* 14: 26. 1924.

<sup>9</sup> Monteith, J. Relation of soil temperature and soil moisture to infection by *Plasmodiophora brassicae* (Abstr.). *Phytopath.* 14: 25. 1924.

<sup>10</sup> Dickson, J. G. Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetii*. *Jour. Agric. Res.* 23: 837-870. 1923.

Dickson, J. G., Eckerson, Sophia, and Link, K. P. The nature of resistance to seedling blight of cereals. *Proc. Nat. Acad. Sci.* 9: 434-439. 1923.

having widely different temperature requirements, the corn seedling being a high-temperature, the wheat seedling a low-temperature plant. What now will happen when seedlings of these two hosts are grown in soil infested by their common parasite and submitted to an experimental range of soil temperatures? The results are surprisingly significant, as illustrated in text figure 3. At the low temperature the wheat seedlings remain healthy,



TEXT FIG. 3. Seedling blight of cereals. A graphic comparison of the influence of incubator temperatures (degrees Centigrade) upon the growth of the fungus *Gibberella saubinetii* with that of the corresponding soil temperatures upon the seedling blights caused by it in corn and in wheat respectively. The fungous growth is stimulated in this case, as with the tomato and tobacco fungi (text figs. 1, 2), to increased growth with rising temperature to an optimum at about 28° C. The significant thing is, however, the sharp contrast in effect of temperature upon the disease-development with the respective hosts, the corn blighting worst at low temperatures, the wheat at relatively high temperatures. Here the influence of temperature upon host seems to be dominant over that on parasite.

while the corn seedlings are invaded and blight. At the high temperatures, conversely, the corn seedlings remain healthy, whereas the wheat seedlings are invaded and blight. Since these investigations were dealing with but one parasite, they were forced to conclude that in this case the explanation must be quite different from that accepted for onion smut. The influence, which with smut may be primarily upon the fungus, must here be primarily on the host. The further studies of Dickson and his associates have not only shown this to be the case, but have gone satisfactorily into the details as to just how the host is modified so as to make it more easily invaded under one environment and to retard the invasion under other conditions. Their conclusions, summarized, are that the embryonic parts of each seedling, corn and wheat alike, are sheathed by a protective covering through which invasion must usually proceed. The cell membranes of this sheath at the outset are easily penetrable, being composed chiefly of pectin. In normally balanced seedling-development, the cell membranes of this protective sheath pass quickly through this pectin stage to a condition of maturity when the celluloses or even lignin and suberin predominate, and

these less easily hydrolyzable wall elements resist the invasion of the fungus. In other words, in the vigorous, normally balanced seedling developing under favorable environmental conditions, the tissues which are subject to invasion pass quickly from a stage of embryonic susceptibility to one of relative resistance. Conversely, any adverse condition which retards normal development correspondingly retards this process and prolongs the state of relative susceptibility to fungous invasion. With wheat, low temperature is essential to normal balanced seedling-development and high temperature disturbs this balance; therefore the wheat seedling is resistant at the low temperature and blights at the high. With the corn seedling, the normal temperature relations are exactly reversed. High temperature is essential to normal balanced seedling-development and low temperature retards this; therefore the corn seedling is resistant at the high temperature and blights at the low. Probing still farther into the details, it appears that the wall changes are but the end results of various metabolic processes involving digestion of reserve foods, with protein as well as carbohydrate changes.

Finally, if soil temperature, in influencing disease, acts here primarily through modifying seedling metabolism, how about the effect of moisture and of light? Dickson's experiments show consistent results with these. In corn and wheat alike, conditions of moisture or light which favor normal seedling metabolism and rapid development inhibit the fungous invasion and consequent disease-development; the reverse conditions aggravate these and increase seedling blight. These results indicate again the intricacies of the problem which one meets when he attempts the experimental analysis of the relation of the environmental complex to the disease complex.

This work with club root, onion smut, and the *Fusarium* diseases includes a sufficient range of types of each parasite and host to illustrate what may probably be expected from further studies upon soil diseases. Evidently, as basic for right thinking, we should accept the idea that soil environment—temperature, moisture, and composition—has a controlling influence upon all soil-borne parasites, whether of the obligate or of the facultative type; and further, that these environmental factors are the ultimate determinants in the broader geographical distribution as well as in the local occurrence and seasonal severity of these diseases.

How easily and completely a single environmental factor may limit the possible geographical distribution of an introduced parasite is illustrated with onion smut. Doubtless of European origin but first described by Frost from New England, this has been carried across the continent to the Pacific with onion sets and established itself successively in each intensive onion-growing center of the northern states. Following the advent of commercial onion culture in the south, literally carloads of smutty onion sets have been taken to Texas and other Gulf states. Yet no smut has developed. Why? Apparently for the simple reason that onion seed is there planted in the autumn while the soil temperature is still above 28° C., the critical upper

limit at which smut spores may germinate and infect. May it not be worth while in connection with the complicated and costly systems of inspection and quarantine to devote more effort to determining what are the natural limits for the harmful spread of possible invaders?

We have thus far cited only examples of soil diseases. Every one recognizes the still more evident relations of aerial environment to the common fungous diseases of foliage and fruit. Quite as obvious are the difficulties of exact experimentation with the aerial factors—especially where light is involved. With modern apparatus, including artificial illumination, as shown by Hottes and by Harvey, these difficulties are being overcome, and we may expect satisfactory future progress with experimentation under controlled air conditions. Here again we shall need to consider, not only the direct influence of each factor, as moisture and temperature, upon the life history of the parasite, spore-germination, and infection, but also the relation of these things to those developments in host tissue which determine the relative susceptibility.

The correlation of these things has been made evident by the studies of Giddings<sup>11</sup> and others with the apple rust (*Gymnosporangium*). The development of the fungus, including the formation of the infective sporidia, seems delicately conditioned upon a favorable combination of climatological factors during a limited period in the spring of the year. At the same time each apple leaf, as it unfolds, passes in succession from a state of early susceptibility to a later one of relative immunity, this progress being under the continually modifying influence of environment.

With the still commoner orchard diseases, apple scab (*Venturia*) and cherry leaf spot (*Coccomyces*), long known in Europe as well as in America, the recent studies of Keitt and L. K. Jones<sup>12</sup> are emphasizing significant new facts in environmental relations. Both diseases alike are caused by ascomycetous fungi which overwinter in the dead host leaves and mature and discharge ascospores for renewed infection in early spring. But the progress of the two diseases, and consequently control measures, are influenced quite differently by environmental factors. These studies show that apple scab is a low-temperature, cherry leaf spot a high-temperature disease. The result is that, under the usual cool, early spring conditions, apple scab proceeds from ascospore discharge to rapidly developing primary and secondary infections, but on the other hand the progress of the disease is retarded upon the advent of summer heat. By contrast, the high-temperature cherry leaf spot develops but slowly in the cool spring, speeds up with rising temperature, and reaches its maximal development with midsummer heat. The relation of these things to a spraying program is obvious. Again this emphasizes that the adequate understanding of the relations of

<sup>11</sup> Giddings, N. J. Infection and immunity in apple rust. W. Va. Agr. Exp. Sta. Tech. Bull. 170. 1918.

<sup>12</sup> Keitt, G. W., and Jones, L. K. Seasonal development and control of apple scab and cherry leaf spot in relation to environment (Abstr.). *Phytopath.* 14: 36. 1924.



environment to disease is not only essential in considering the fundamental relations of parasite and host, but they are equally basic to the practical problems, disease-distribution, severity; and control.

And finally we need constantly to remind ourselves as plant pathologists that all these problems of environment are problems of physiology. Looking backward, we acknowledge the great debt of plant pathology to mycology, including bacteriology. The germ theory of disease, plant and animal, has given to these mycological branches of biology much of their activating stimulus, their great opportunity, their adequate support for a generation past. Do not we phytopathologists, generally better trained in mycology than in physiology, owe it to the coming generation to stimulate and support the physiological developments until a more normal balance is reached? Should we not wisely remind ourselves that in human pathology such serious lack of balance has never obtained? The reason is simple. Most of the researches in human physiology have traditionally proceeded in the medical faculties. The need in human pathology of the constant association of the physiological with the mycological have continued so evident that no segregation in their relative progress has occurred. The keenest young men trained in general physiology have found cordial support and welcome opportunity for directing their talents to the physiological aspects of animal pathology. In this spirit of correlated endeavor animal physiology and pathology have been making their splendid progress, probing ever deeper into the fundamentals and ever bringing from these more aid in the applications. May we profit from the comparison? Have we as plant pathologists been as ready in the past as we should be in the future cordially to welcome promising young plant physiologists to join our research groups, to share our problems, our laboratories, our experimental houses, and, most important of all, our budgets?

THE UNIVERSITY OF WISCONSIN



## RECENT ADVANCES IN CYTOLOGY<sup>1</sup>

LESTER W. SHARP

The acceptance of an invitation to outline for you in a few minutes the recent advances in cytology places me between two hazards characteristic of such a situation. On one hand is the danger of making statements so general and obvious that they would be devoid of new meaning and therefore wasteful of your time, and on the other is the risk of presenting an arid recital of bare facts equally unkind to your patience. The difficulty increases when one inclines toward a definition of cytology broad enough to include any work in which the cell is more or less directly studied with scientific method, no matter what the special avenue of approach; and when one regards as advances not simply the establishment of new facts or the attainment of certain temporary goals, but also the consideration of old facts from new points of view, the recognition of errors, the invention or adaptation of new methods, the formulation of new and useful provisional hypotheses, the correlation of neighboring fields of research, and the confident entry upon new paths of endeavor. Neither in generalities nor in particulars alone can recent cytological progress be represented. I shall therefore follow a somewhat ill-defined middle course by limiting my account to a very few of the developments which cytologists have recently witnessed in their field, with some indication of their reasons for considering them of sufficient significance to be brought before you on this occasion.

*Protoplasm.* I can think of no problem of more fundamental importance to cytology, and to all biology, than that of the nature of the physical basis of life. After the masterly addresses of Professor Harper at Pittsburgh and Professor Wilson at Boston I feel that comparatively little remains for me to say on this subject, but it may not be superfluous to emphasize anew the impetus which the conception of protoplasm as a colloidal system has given to all types of cell study.

In the first place, it has encouraged a more thoroughgoing investigation of the cell in the living state. This has involved the devising of ingenious methods by which we have already been able to remove many misconceptions originally growing out of the too-exclusive use of fixed material.

Again, by furnishing us with a new and suggestive theoretical basis of interpretation for the data obtained from morphological and physiological cell studies, it has aided us in finding plausible partial explanations for a variety of puzzling phenomena, among which we may mention cytokinesis,

<sup>1</sup> Invitation paper read at the joint meeting of Section G, American Association for the Advancement of Science, the Botanical Society of America, and the American Phytopathological Society at Cincinnati, December 28, 1923.

the achromatic figure, permeability, the localization and reversibility of reactions, and growth. This has inspired us with confidence in our ability to make further progress with our problem, but we must beware of thinking the problem too simple, and never lose sight of what explanation really means in science.

Furthermore, the new conception has removed much of the apparent force from theories postulating ultimate vital units in protoplasm. It is very doubtful if such a theory as that of the late Professor Meyer, for example, notwithstanding its just claim to the most careful consideration with regard to certain of its aspects, can longer be of direct service to the biologist, who now feels more than ever constrained to regard the physical basis of life as a complicated system of active constituents, and not as a colony of special living units fundamentally distinct from the ergastic components of the cell as Meyer so strongly contended.

Finally, the colloidal theory has brought into closer association on a common ground the cell morphologist, the physiologist, and the physical chemist. Here without doubt is one of the most noteworthy recent developments in which cytology has shared, for only in the united efforts of at least these three is there any hope of coming nearer to a solution of this most complex and baffling of cytological problems—indeed, without some measure of correlation cytology would not now be enjoying the benefits already derived from the use of the colloidal theory. We can now formulate our problems more explicitly than before, and there is often as much progress denoted in the adequate statement of questions as there is in their answers.

*Cytoplasmic Inclusions.* Narrowing our attention to certain inclusions of the cytoplasm, we find ourselves confronted with an array of questions which have arisen largely as a result of the recent intensive study of chondriosomes. Our progress here has consisted chiefly in defining more clearly the points to be determined, although a number of preliminary questions have received answers. It has been shown that plastids develop from minute primordia in young cells. It has also been shown that the cell contains a series of small bodies, usually called chondriosomes, which are in some way concerned in the formation of a variety of metabolic products. Among the questions to which answers are now being sought, three may be cited because of their immediate and obvious importance:

First, are the aforementioned two classes of bodies wholly distinct or not? In other words, are the plastid primordia actually chondriosomes of some special kind, or do they merely resemble them because of their small size? At present the evidence appears to favor the second of these alternatives; surely plastids are distinct from at least some of the cell inclusions to which the name *chondriosome* has been applied. Only one who has worked with these minute objects can sense the extreme technical difficulties which here block the path to certainty.

Second, however we answer the preceding question, what is the ultimate

origin of those bodies which become plastids? Do they arise only by the sort of division they are later seen to undergo throughout their development into plastids, or do they differentiate anew under certain conditions in the cytoplasm? In the maize plant they have been traced back to the limit of microscopic visibility. We have here the appearance of origin *de novo*, but only as regards visible objects; what is their history, if any, prior to the moment they attain visibility? We do not know, but I am of the opinion that cytologists have often underestimated the capacity of protoplasm for epigenetic differentiation, and have thus been too averse to the idea of origin *de novo*.

Third, what is the true status of the bodies functioning otherwise than in plastogenesis? Are any of them actually protoplasmic organs with specific rôles in the elaboration of ergastic products, as one school strenuously contends, or are they all, as they seem to be in part, the ergastic materials themselves in process of elaboration and utilization by the cytoplasm? Division is frequently cited as a reason for ranking them as organs, but the evidence on this point scarcely permits us to say that we are dealing with the active multiplication of autonomous units. On the other hand, simple ergastic cell inclusions would hardly be expected to behave as do the chondriosomes in the animal spermatid.

To the cytologist, this continued uncertainty regarding the genetic continuity of plastids and their relation to other cell elements is very annoying; to the geneticist who is seeking to explain the peculiar behavior of certain inherited characters showing themselves in cytoplasmic organization, it is especially exasperating. But so the matter stands. What is urgently needed is a knowledge of the cytoplasm at all stages of the life cycle at least as full as that which we have of the nucleus. Until we obtain it we may expect much of the troublesome uncertainty to remain.

With regard to other cytoplasmic inclusions, the past few years have witnessed an interesting renewal of the study of vacuoles in plant cells. The positive results of this study are as yet rather meager, but their suggestive nature leads us to anticipate noteworthy developments in our knowledge of the true history of these elements and of their relation to other cell components, particularly the Golgi material of animal cells. Mention of the Golgi material brings to mind one of the most notable advances recently made in the investigation of cytoplasmic elements in the cells of animals. After being perplexed for many years by conflicting and unconvincing accounts of the behavior of certain obscure bodies during spermatogenesis, we have now been given a particularly illuminating description of this process in insects and amphibians. The history of the nucleus, centrioles, Golgi material, and chondriosomes has been traced with such extreme care from spermatocyte to spermatozoön that we believe we have at last a trustworthy story of this most complicated series of cell transformations, and a basis of interpretation for similar studies in other animal groups.

*Chromosomes.* It goes without saying that the development in modern cytology which has created the greatest widespread interest is that concerning the mechanism of Mendelian heredity. This general situation is so well known that it is almost superfluous to do more than mention it before this audience. The briefest of summaries of what the cytologist has really established regarding this mechanism may nevertheless be of some service.

In an ordinary higher organism, the diploid chromosome complement of each somatic and early germ cell consists of two similar intermingled sets of chromosomes derived by repeated equational division from the haploid sets contributed by the parents through the gametes at the previous fertilization. In many cases the chromosomes of each set differ visibly from one another, the degree of such differentiation varying widely in different organisms. At the time of meiosis, if not earlier, each chromosome of one set pairs with the corresponding one of the other set, and the two segregate in the reduction division independently of all the other pairs, so that each resulting gamete (or spore, followed later by the gamete in plants) contains a haploid set made up of one member of each of the pairs. A multitude of cytological observations and breeding experiments have revealed a remarkable parallelism between this distribution of chromosome pairs through development, reduction, and fertilization and that of independent Mendelian character pairs, all doubt that the parallelism is complete having been removed by exhaustive researches on heteromorphic chromosomes in certain orthopterans. The time for questioning a causal significance in this parallelism, originally suggested twenty years ago, has long since passed.

The fact that an organism has so many more pairs of allelomorphic characters than of chromosomes has led us to infer that each chromosome must have a specific or differential effect upon a number of characters, and that the chromosomes of the complement are as differentiated functionally as they frequently are in form. What we know of linkage groups, notably in *Drosophila*, assures us of the safety of these inferences. This brings into the front rank of importance the long-debated question of chromosome structure. We have been greatly interested to find that in certain insects the chromatic lumps or chromomeres show a remarkable constancy in size and position in a given chromosome of the complement, and that constrictions, chromomere vesicles, and points of fiber-attachment also have definite and characteristic locations. The obvious significance of these facts is that they seem to indicate a longitudinal differentiation in the morphology of the chromosome; further than this we can scarcely go at present with their evaluation. By both the older methods and the very promising aceto-carminic process we have discovered evidence that the chromosome is a compound body, but we are not yet in a position to assert that the visible parts of which it appears to be composed are autonomous, or that they represent regions with different functions in heredity. We strongly hope that such is the case, but this should make us the more

critical of our evidence. Let nobody be so enthusiastic as to say that the cytologist has identified the postulated hereditary units of Weismann or of anyone else.

Beyond this point the cytologist has as yet established disappointingly little concerning the structure and normal behavior of chromosomes in which he feels that he can ask the geneticist to place unrestricted confidence. Because of the shifting nature of our views regarding the architecture of the chromosome, it is impossible to state precisely what is involved in its division. With reference to the mechanism of crossing over, we can at least be sure that parasynapsis occurs, a point placed beyond all doubt by recent researches on certain orthopterans and worms. But we are not at all certain of its universality; the evidence at present available indicates another mode of conjugation in *Oenothera*, to which so much genetic interest attaches, though I suspect that there may be more to learn on this point. What occurs between the synaptic mates during conjugation—whether or not there is an actual recombination of parts as was at one time suggested on cytological grounds—we must be frank to admit that we do not know. In spite of careful investigation and the extremely serviceable nature of the conception, it has not been demonstrated, and cytologists are becoming less and less inclined to think that it occurs.<sup>2</sup> This is another matter in which desire easily influences judgment. We are constantly reminded of the genetic evidence for chiasmotypy, but we must not forget that other logically prior hypothetical postulates are here involved. This is not to undervalue the advantage which cytology has derived from genetics; the cytologist must continue to make the most of the light shed upon his path by hypotheses suggested by his colleagues. I venture to think, however, that he is still justified in his contention that statements of fact regarding chromosome structure and behavior are trustworthy only if they are founded primarily on cytological evidence. Progress in our knowledge along these lines may indeed be what has been called plodding; but by this method of procedure our feet are kept on solid ground, and it is highly essential that the foundations for our genetic theories be solidly laid.

We have recently received a new suggestion regarding the manner in which regions of chromosomes may become interchanged. Citing reported cases in which chromosomes, after becoming temporarily fragmented, reassociate at meiosis, Prell<sup>3</sup> points out that, should the parts occasionally recombine in new ways, chromosomes with new constitutions would result, much as after the process of chiasmotypy. This supplementary hypothesis of rhegmatypy, as it is called, we shall do well to bear in mind, especially when dealing with organisms in which evidence for parasynapsis is lacking.

Nothing is more reassuring in cytology at the present time than what we are able to ascertain concerning the rôle of chromosomes from their oc-

<sup>2</sup> This was written before the appearance of the recent important paper by Janssens.

<sup>3</sup> Prell, H. Die Theorie der Rhegmatypie. *Genetica* 5: 177-190. 4 text figs. 1923.



casional misbehavior. In such phenomena as non-disjunction and polyploidy nature performs almost before our eyes a series of experiments from which we should be dull indeed were we to learn nothing. It is only necessary to cite what has been observed regarding the relation of chromosome aberrations to certain classes of mutations, as for example in *Datura*, and the way in which the triploid *Drosophilas* have helped to revise our early naïve notions concerning the connection between chromosomes and the state of the organism we call sex. We have almost everything to learn about the causes of such aberrations, but it is already clear that they play a part in the production of new races; and when we consider the multiploid series of species in many genera and families we can have little doubt that they have functioned in the origin of species also. Moreover, the frequent accompaniment of polyploidy by apogamy, the relation of both of these to hybridization, and the behavior of chromosomes in known and suspected hybrids, have given us not only a cytological test for hybridization, but also a far deeper insight into a variety of perplexing situations, such as aberrant genetic ratios, the instability of some hybrids, the curious constancy of others, sterility, and the polymorphism of many natural groups. It is here that cytology is being brought into an alliance with taxonomy, an alliance which neither can afford to shun as entangling.

The study of chromosomes and of their rôle in heredity has thus yielded results of the highest import. We have been fortunate in having working hypotheses of extreme usefulness and adaptability, but this is no reason for forgetting the distinction between true verification and that rationalizing process by means of which we find arguments for what we already believe, or have been told that we ought to believe. Although this process has its legitimate uses, we must be cautious regarding the extent to which we allow it to function in the search for truth in cytology.

Time does not permit consideration of many other fruitful lines of endeavor in cell biology, such as researches on the structure of protozoa and protophyta, tissue cultures, and the relation of the hydrogen-ion concentration of cells to their many reactions, including staining. After the manner of a committee, we may report substantial progress and promise that the investigations will continue.

*The Cell Theory.* There is one further matter of general biological interest which I trust I may be permitted to mention briefly and without the discussion it merits. It concerns the relation of the cell to the multicellular organism of which it is a part.

Ever since the foundation of the cell theory there has been a persistent tendency, at least among botanists, to remain more or less with Schleiden and Schwann in regarding the cells as the primary agents in development, and the organism as little more than an aggregation of coöperating cell individuals. This elementalistic point of view has been maintained in spite of telling arguments favoring the plasmodial theory, namely, that at

all stages it is the organism as a whole that is the primary agent of organization, and that development consists essentially in the incomplete subdivision of a growing and differentiating mass of protoplasm into subordinate specialized parts, the cells. It seems to me that one can not long contemplate what cytologists, histologists, and embryologists have recorded concerning modes of protoplasmic division as related to the fate of the resulting cells, the genesis of tissues, protoplasmic connections, the reactions of blastomeres, and a variety of other phenomena, without feeling that the latter view is in the better accord with our observations, and that De Bary was almost certainly correct when he said that the organism builds the cells, rather than the reverse.

This view has a number of phylogenetic implications, perhaps the most obvious being that a considerable share of the importance hitherto attributed to colonial forms actually belongs to the much-neglected coenocytes. Furthermore, I think it removes all force from the frequently heard objection that the conception of the organism as a whole is too mystic for biology to work with. Surely a continuous but differentiated mass of reacting protoplasm will be found no more difficult of comprehension than a cell state.

*Conclusion.* To sum up, we may say that cytology has recently shown advance (1) in the discovery of new facts, some of which, notably those pertaining to chromosomes, are of prime significance; (2) in giving to the extranuclear portion of the cell some of the attention it has long deserved; (3) in methods, which we feel confident will enable us further to rectify errors and establish new groups of facts; (4) in becoming more experimental, which should not, however, lead us to disparage the disinterested observation and purely descriptive work which will long be of value in all sciences, particularly in biology; (5) in its use of new and effective working hypotheses or bases of interpretation, some of which have come from other fields; and (6) in the definite and profitable alliances which have developed with other branches of biology and with the other sciences.

By all these signs of progress, especially the last, we feel encouraged for the future of cytology. We have long realized that all biological problems are to some extent cell problems, and that the latter are in some measure problems of physics and chemistry. What encourages us is the fact that we can now more clearly discern the nature of the common ground on which these departments of science naturally meet. This warrants us in confidently expecting that we shall bring them into complete integration, which is as essential to the welfare of biology as it is to biology's subject, the living organism.

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## STUDIES IN THE LIFE HISTORY AND PHYSIOLOGY OF CERTAIN SMUTS

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### INTRODUCTION

Smuts have been known for a long time as obligate parasites which develop their vegetative mycelium and chlamydospores on living hosts. Presumably, if the conditions of their parasitic life could be established under laboratory conditions, they would complete their life history equally well on artificial media. To determine what such conditions might be, selected species from material obtainable were subjected to scrutiny as to their reactions to special types of nutrition, to stimulation, temperature, and other factors. In the course of this study, morphological details in the life history of the selected smuts were also worked out as far as possible. The following species were studied: *Tilletia Tritici* (Bjerk.) Wint.; *Tilletia foetans* (B. & C.) Trel.; *Ustilago Tritici* (Pers.) Rostr.; *Ustilago Hordei* (Pers.) Kell. & Sw.; *Ustilago Zeae* (Beckm.) Unger; and *Ustilago Heufleri* Fuckel.

Since the appearance of Brefeld's (2) works, very little attention has been given to the cultivation of the smuts. Brefeld germinated the chlamydospores of a large number of species and kept them going in nutrient solutions, thus demonstrating in part their saprophytic possibilities. Here they multiplied by repeated and abundant formation of secondary spores, and the mycelium was either scanty or not formed at all. In some cases, however, e.g., *Tilletia*, in old cultures, Brefeld observed chlamydospore-like cells but did not test their capabilities. Speaking of *Ustilago Zeae*, Rawitscher (17, p. 686), in reviewing the situation, states:

Die Sporidien vermehren sich massenweise durch Sprossen, aber sie kopulieren weder (siehe darüber auch Brefeld), noch bilden sie richtige Mycelien . . . selbst in festen Nährböden.

As will be shown later in this paper, the mycelium of the corn smut can be developed and can be kept growing continuously on artificial media.

Potter (16) reports that *Sorosporium reilianum* (Kühn) McAlpine grows readily on solid nutrient media, but that "its growth is almost exclusively conidial under favorable conditions."

Knip (12) carried through the first complete cycle from chlamydospore to chlamydospore on artificial media. He grew *Urocystis Anemones* (Pers.) Winter in a 0.1 percent malt-extract solution, and obtained chlamydospores in a few weeks' time. He considers that the saprophytically grown

chlamydospore is morphologically identical with the smut spores derived from the host. No evidence, however, appears in Kniep's paper of the ability of the spores so grown to germinate and to form the usual promycelium and sporidia. He points out, furthermore, that this malt-extract solution does not bring about the same results with some other Ustilaginales tested. From a series of concentrations of malt extract he found that a 0.5-percent solution is the optimum concentration for mycelium-formation, and it can be assumed that this smut can be grown in this way, in the vegetative condition, for any length of time.<sup>1</sup>

#### INFECTION EXPERIMENTS

##### *Tilletia Tritici* (Bjerk.) Winter

The details of germination will not be taken up in this paper; numerous studies have appeared since the first account of it. Concerning infection, we have the earlier work of Kühn (13) and Wolff (21), which, although significant and thorough for the time, needs modern verification. Brefeld (2) studied the development of *Tilletia* in water and nutrient solutions. Dangeard (3), Rawitscher (18), and Paravicini (15) have given accounts of the nuclear phenomena. Most of the other work on *Tilletia* has been concerned with the control of the disease which it causes.

A logical procedure for the study of a fungous parasite is to determine how it enters the host. With this in mind, experiments were devised which would throw additional light upon the manner of infection of wheat by *Tilletia Tritici*. This smut has three kinds of spores: chlamydospores, *i.e.*, the spores that are produced on the plant; sporidia, formed at the tip of the promycelium; and secondary spores, formed by the fused sporidia. The nuclear condition of the spore is of importance, since the completion of a nuclear process may have a bearing on the time at which the chlamydospores can germinate. According to Rawitscher (18), the original fusion nucleus divides, not only twice, but three or more times within the chlamydospore, so that the promycelium has 8 or more nuclei, the sporidia receiving each one nucleus, and the fusion of pairs of these sporidia resulting in a binucleated germ tube.

In order to determine the time of infection, 1400 seeds were sterilized and potted out, a hundred to the pot. A susceptible variety of wheat, Washington Hybrid no. 128,<sup>2</sup> was used in all the infection experiments. Each successive day the seeds from one pot were removed, dusted with chlamydospores, and transplanted to the field. This was continued until the leaves of the wheat came out of the sheath, and then plats of wheat of the same age

<sup>1</sup> The other literature concerning the smuts has been summarized by various authors in several connections. Papers which are pertinent from the standpoint of the writer's investigation are especially those of Kniep (12), Rawitscher (17, 19), Lutman (14), Brefeld (2), Potter (16), Stakman (20), and Paravicini (15).

<sup>2</sup> Washington Hybrid 128 was sent to me by Prof. F. D. Heald of the State College of Washington, Pullman, Washington.

were dusted with spores until the plants were five or six centimeters high. The results of the experiments are given in table 1.

TABLE 1. *Infection of Wheat by the Chlamydospores of Tilletia Tritici*

Day Dusted	Smutted	Sound	Percent Smutted
1	93	0	100.00
2	94	0	100.00
3	90	0	100.00
4	82	11	88.16
5	70	24	74.46
6	22	64	74.42
7	10	78	11.36
8	10	81	10.98
9	2	87	2.25
10	2	89	2.20
11	0	93	.00
12	0	86	.00
13	0	91	.00
14	0	92	.00
Control	0	93	.00

The table shows that there is little or no infection when the wheat seedlings are dusted with chlamydospores ten to twelve days after planting. This is about the time it takes the leaves to grow out beyond the sheath. The greatest number of plants are infected when the seeds and the smut spores are planted together, and for the first three or four days after planting. Results indicate that infection takes place at the time of germination and diminishes rapidly after the sixth day.

A similar experiment was performed, in which only the sporidia were placed upon the wheat and wheat seedlings. The chlamydospores were germinated in pots of sterile soil or on agar media in Petri dishes. Large numbers of chlamydospores were dusted upon the surface of the medium used for germination, and the containers were placed in a saturated atmosphere. If spore balls are selected with care and broken by rolling between the sterilized thumb and index finger, allowing the spores to fall upon sterile paper, spores of such purity can be obtained that there will be very few contaminations on agar or on sterile soil. When large masses of spores germinate, the promycelia are erect and the sporidia are borne above the substratum. It is then a comparatively easy matter to place sporidia upon the wheat seeds. Moist sterile wheat seeds were picked up by means of sterile forceps and touched very lightly to the mass of sporidia; or a camel's-hair brush was used to apply the sporidia to the seedlings. The grains of wheat and the seedlings were examined under the low power of the microscope to make sure that there were only sporidia present. The results of the experiments are given in table 2.

The results can be seen to imitate closely those obtained in the first experiment.

TABLE 2. *Infection of Wheat by Sporidia of Tilletia Tritici*

Lot of 1,400 seeds planted on the same day: 100 seeds dusted with sporidia on successive days. Wheat; Washington Hybrid 128.

Day Dusted	Smutted	Sound	Percent Smutted
1	91	0	100.00
2	94	0	100.00
3	93	0	100.00
4	86	9	90.63
5	80	12	87.00
6	73	16	82.00
7	60	32	63.04
8	63	31	65.00
9	40	55	42.10
10	12	81	12.90
11	3	86	3.37
12	2	89	2.20
13	0	94	.00
14	0	90	.00
Control	0	93	.00

A third experiment was performed to determine whether or not the secondary spores and the mycelium could infect the host. A large amount of secondary spores and mycelium was obtained by making single-spore cultures from sporidia on nutrient-agar media. Many of these cultures were made in 500-cc. Erlenmeyer flasks, and, when they had developed a vigorous growth of mycelium as well as secondary spores, sterile wheat seeds were planted on the cultures. The seeds germinated and grew in the culture, and the mycelium developed around the wheat seeds and even grew up the hypocotyl to form a white mat of mycelium around the sheath (Pl. XXXIX, fig. 4). Every week some of the seedlings were transplanted to the field. The results of the experiment are given in table 3.

TABLE 3. *Effect of Saprophytic Mycelium on Wheat Seedlings*

Days in Culture	Seeds	Plants Smutted	Plants Sound
7	100	0	94
14	250	0	233
21	200	0	181
30	200	0	186
Control	100	0	88

Further infection experiments with mycelium were set up as follows:

(1) Bits of the mycelium were placed on 300 sterile wheat seeds at the time of planting. All the plants produced sound grain.

(2) The mycelium was placed on seedlings 1 to 20 days old. The plants were covered with battery jars during the first 48 to 72 hours, to insure a saturated atmosphere. All the plants produced sound grain.

The results of the experiments show that the secondary spores and the mycelium are not able to infect the host, at least at the time when the host, according to other results, is susceptible.

A great number of both free-hand and microtome sections were made of the plants that had the mycelium and secondary spores growing on them, and in no case, not even when the plant was completely covered by the mycelium, was the fungus found to enter the host. It is evident that the secondary spores and the mycelium which has been grown in culture are not able to infect the wheat plants.

The experiments with the three different kinds of spores of *T. Tritici* show very clearly that germ tubes of sporidia are instrumental in entering the host, and it is also evident that the infection takes place only at the time of germination or within a few days following.

Now the questions arise: Just how do the germ tubes from the sporidia enter the host? And at what point do they enter? To answer these questions the following method of experimentation was adopted. Wheat seeds were sterilized, dusted with chlamydospores, and planted in pots of sterile soil. Metal pots, 14 × 6 × 6 inches, one side of which could be removed, were filled with moist soil, sterilized, the side was removed, the seeds were planted on the surface of the soil, and the side was replaced. The pots were set in such a manner that the side on which the seeds were planted was in a vertical position. The advantage of this arrangement was that the conditions of growth, both for the plant and for the fungus, were favorable, and that the seedlings could be removed almost free from soil. The seedlings were mounted under the low power of the compound microscope and carefully examined.

It was found that some chlamydospores always cling to the sheath, and germinate to form a promycelium which bears sporidia at the tip. The promycelium of *Tilletia Tritici* is negatively geotropic, as it always grows upward. The sporidia are pressed tightly against the sheath and the promycelium grows up in contact with the plant. With an 8-mm. objective one can see the fused sporidia and the very fine germ tubes coming from them. The germ tube may grow along the surface of the sheath for two or three hundred microns before it enters the host. It penetrates the epidermis of the sheath, grows in between the cells, and pushes them apart, the host offering very little resistance (Pl. XL, figs. 6, 7 *a*). It is very seldom that the germ tube enters the cells of the sheath (Fig. 7 *b*), and therefore the mycelium in the sheath and in the root crown is intercellular. As soon as the tip of the germ tube enters the host, the part at the surface withers away. One must, therefore, find the tube just at the time it is entering if the connection with the sporidia is to be seen. The best time for observing this is from six to eight days after planting, if the seeds have been planted in moist soil and the temperature is about 30° C. It can also be seen very well if germinated chlamydospores are placed on seedlings 2 to 3 days old, and sections are made 50 to 80 hours afterwards.

### *Ustilago Zeae* (Beckm.) Unger

Since the mycelium of *Tilletia Tritici* could not infect the host, it became a matter of interest to determine whether or not the mycelium of *Ustilago Zeae*, which had been obtained in culture, could infect the corn plant. *U. Zeae* was used for comparison because both Lutman (14) and Brefeld (2) have shown how the germ tube enters the host. If the sporidia of *U. Zeae* are placed on a glycerin agar, they will conjugate and form a binucleate mycelium (Pl. XXXIX, fig. 3). In a culture of this kind there are many round bodies formed that resemble chlamydospores (Pl. XL, fig. 8 c). The following experiments were performed with the mycelium:

(1) Corn was grown under sterile conditions in large 12-inch test tubes, and the mycelium was placed on plants of various ages.

(2) Plants were grown in the greenhouse, and the mycelium was placed on the growing points of the plants and on the young flowers. The young plants were placed in a saturated atmosphere for 48 hours; the growing points of the older plants were kept moist by means of absorbent cotton.

(3) In another experiment, pieces of sterile maize leaf were floated on a 5-percent solution of dextrose. Sporidia under the same conditions attached themselves to the leaves and formed a germ tube that entered the host as already described by Brefeld and Lutman.

In all these experiments the mycelium did not enter any of the plant tissues, thereby showing conclusively that the mycelium obtained in culture is unable as such to attack the host.

### *Ustilago Heufleri* Fuckel

*Ustilago Heufleri* is parasitic on *Erythronium americanum*, and is one of the smuts that is very local in its distribution. It is found near Ann Arbor, in only one or two places, and it seems not to have spread beyond the very limited area where it has been known to occur for some years. The smut spores used in this study were collected in May, 1922, and the bulbs of *Erythronium* in October, 1922. The bulbs were collected in a wood one mile west of Ann Arbor, where the disease has never been known to occur. The bulbs were potted in soil, and were kept out of doors throughout the winter. When the weather became warm, the bulbs were removed to a refrigerator that was kept at a temperature of 3° C. These bulbs and smut material were used in the following experiments.

Twenty bulbs were dusted with chlamydospores at the time of planting, March 14, 1923. On May 3, sixteen of the plants of *Erythronium* had become diseased, showing pustules of chlamydospores on the leaves. The leaves of the eight plants used as controls showed no signs of infection.

In order to see whether the smut could attack the leaves of *Erythronium* when they were above ground, 160 bulbs were planted March 1, 1923, and when the leaves were about 2 cm. above the ground they were sprayed with



chlamydospores. The pots were placed in a saturated atmosphere for 48 hours, and then were placed in the greenhouse. On March 18, the greenhouse, which is usually heated to 65° F., became heated to a much higher temperature, and all the plants were killed. Up to that time, however, none of the leaves had shown any signs of infection.

A little later in the year, when the plants could be kept out of doors, the experiment was repeated. 25 bulbs were planted March 14, 1923, and were placed out of doors to grow. On May 11, when the leaves were about 2 cm. high, they were sprayed with chlamydospores, placed in a saturated atmosphere for 48 to 72 hours, and then put out of doors on the north side of the building. All the plants remained healthy and showed no signs of infection.

On May 2, 1923, twenty *Erythronium* plants were sprayed with secondary spores that had been growing in culture since November, 1922. The sporidia were transferred from the culture to a 5-percent solution of malt extract, and when they were budding rapidly they were placed on the leaves of the plants. The plants were kept in a saturated atmosphere for 48 to 72 hours, and then were placed out of doors on the north side of the building. None of the leaves became infected by the smut. This experiment shows that the secondary spores are not able to enter the leaves of the host, when the leaves are above ground.

Bits of sterile leaves of *Erythronium* were floated on 5-percent solutions of dextrose and cane sugar in which the secondary spores of *Ustilago Heufleri* were budding. The presence of the pieces of leaves caused a rapid budding of the secondary spores, and soon large numbers of them attached themselves to the surface of the leaves. Observations were made every day with an 8-mm. objective. Under the conditions described, the secondary spore forms a short germ tube at one end, and the tube enters the epidermis of the leaf before it becomes very long. Usually it is so short that it appears as though one end of the spore were pushed in between the cells. No appressorium is formed, but the cell walls are spread apart, the opening taking on a funnel-like appearance. The entrance is very similar to that of *Ustilago Zeae* as described by Brefeld (2). Observations are best made in free-hand sections of the leaves that have been infected by the secondary spores. After the germ tube has passed between the cells of the epidermis, it forms an intercellular mycelium that fills the intercellular spaces, crowding or pushing the cells apart. It is very infrequently that the germ tube enters a cell or through a stomatal opening. Large numbers of secondary spores were found attached to the cut edges of the leaf. The tubes had entered the cut or broken cells or pushed in between the cells, and soon formed an intercellular mycelium. The cultures did not last long enough to form spores, but in some cases the mycelium enlarged and broke up into segments. The segments, however, never rounded up nor became as large as mature chlamydospores, but merely became dark and formed rather heavy walls.

This experiment suggested that the germ tube might be able to infect



the leaves of *Erythronium* through wounds. The leaves of twenty plants were wounded by cutting out a small piece of the leaf or by lifting the epidermis by means of a sharp razor blade. The sporidia and secondary spores from a culture were placed on the wounds, and some of the pots of plants were placed in a saturated atmosphere for 48 to 60 hours: in other cases the wounds on the leaves were covered with paraffin. All the pots were placed out of doors. None of the leaves showed any signs of infection.

Two experiments were performed with the mycelium of *Ustilago Heufleri* that was growing in the tissue of the host. (1) A small cut was made in a healthy leaf of *Erythronium*, and a bit of the leaf that had been infected in the field was placed under the epidermis. The wounds were sealed over with paraffin, or the *Erythronium* plants were placed in a saturated atmosphere for 48 to 72 hours. (2) Portions of leaves that had been infected in the laboratory were placed under the epidermis of a healthy leaf. Both experiments gave negative results.

The results of the experiments indicate that the fungus infects the plant at the time when it is coming up through the soil, and that the leaves that are above ground are apparently able to resist the attack of the parasite. The germ tubes from the secondary spores are able to infect pieces of leaves that are floating on a sugar solution and which are probably of a greatly lowered vitality, but are apparently not able to enter a healthy growing leaf, even when the leaf has been wounded. The mycelium under the conditions of the experiment seems unable to continue its growth when transferred to a healthy leaf.

To sum up: *Tilletia Tritici* infects the young plant before the leaves break out of the sheath. The germ tube from the fused sporidia enters the sheath and forms an intercellular mycelium. Neither a germ tube from a secondary spore nor the mycelium is able to infect the host. The uninucleate sporidia and secondary spores of *Ustilago Zeae* form tubes that can infect the host at any young growing point of the plant, as shown by Brefeld (2). *Ustilago Heufleri* infects the host at the time it is growing up through the soil, and it is unable to attack leaves that are noticeably above ground.

#### CULTURAL LIFE HISTORY OF *TILLETIA TRITICI* AND *TILLETIA FOETANS* ON AGAR MEDIA

Brefeld (2), Stakman (20), and others who have cultivated these smuts germinated and grew them in water or in nutrient solutions. The account of Brefeld is masterly in detail and quite complete as far as it goes, and has been the classic source of information ever since his work appeared. It would seem, however, worth while to give again—this time under different cultural conditions—the story of the life history of each of these fungi as it runs its course on solid media of highly favorable nutrient constitution.

Pure cultures were obtained by means of the dilution method, or by the Kauffman spray method (10) for the isolation of a single spore. A clear

gelatin containing 2-percent malt extract or 5-percent sucrose was used in all the isolation work. The malt-extract medium is the better for general work. Single spores were transferred to the nutrient agars, and to nutrient solutions of the kind hereinafter described. The stock cultures were kept on oatmeal agar in six-inch test tubes.

These two smuts are alike in their behavior. Only the results of the experiments on *T. Tritici* will therefore be given. The chlamydospores of *T. Tritici* germinate within 2 to 3 days when placed on 2-percent malt-extract agar; under these conditions they form a non-septate promycelium, at the tip of which is borne a whorl of sporidia. There are usually eight sporidia, but the number may vary from 1 to 16 or more. The sporidia nearly always fuse in pairs before they become free from the promycelium, and may form either germ tubes or secondary spores. Two or three weeks after the germination of the chlamydospores on oatmeal agar, a mycelial layer appears. This spreads until it covers the surface of the agar by its radial growth (Pl. XXXIX, figs. 2, 4). It is white at the surface of the layer and of a fine leathery texture. It is about 0.5 to 1 cm. in thickness at the center of the mat; here also it is thickest and forms a peak. Beneath the surface, the mat is made up of a mycelium that is yellowish to light brown. After three or four months the culture takes on a brown to dark brown color, and drops of moisture collect at the surface, which has become uneven. The culture now has the characteristic odor of the stinking smut.

The mycelium rises from the fused sporidia as noted above. At first the sporidia are non-septate and are densely filled with protoplasm containing few vacuoles. They usually fuse in pairs before they are detached from the promycelium; if a single sporidium is detached it fuses with its nearest neighbor, but if too much isolated it is unable to fuse with another in the usual way. One of the pair of the fused sporidia soon sends out a slender germ tube from near its tip. At first the tube is entirely filled with protoplasm derived from the sporidia. The germ tube becomes very long, narrow, and often much coiled. It is filled with protoplasm at first, but eventually becomes empty and septate from the base outward, retaining protoplasm only at the somewhat swollen tip (Pl. XL, fig. 5 *d*). As the germ tube increases in length, the protoplasm of the sporidia first becomes vacuolate and finally disappears altogether (Pl. XL, fig. 5 *c*). At the same time 5 to 7 septa appear. The germ tube now continues to grow, with most of the protoplasm at the distal end, while cross walls appear successively on the older and lower portions, where the cells seem to have a very slight amount of protoplasm. Finally the walls of the germ tubes disintegrate.

Often, however, the fused sporidia send out short, thick hyphae instead of germ tubes (Pl. XL, fig. 5 *d*). The hyphae may have a diameter of 15 to 20  $\mu$ , and their tips are swollen and filled with protoplasm. At the base, where the hypha is attached to the sporidium, it is narrow and later becomes

septate and empty. At other times, the fused sporidia may produce crescent-shaped spores which are up to  $25-45 \times 4-6 \mu$  (Pl. XL, fig. 5 c). The secondary spores are attached to the fused sporidia by very slender filaments resembling sterigmata. These secondary spores send out germ tubes or hyphae that continue to form more secondary spores. The germ tubes may also branch from the swollen tip and produce secondary spores on the branches. Often a secondary spore simply elongates into a thick filament; at first this retains the shape of the secondary spore, but later it straightens out and the protoplasm moves to its tip, while the empty region is cut off by cross walls. As the protoplasm continues to move towards the tip, the empty portion becomes multiseptate and finally disintegrates.

The large filaments formed by the sporidia and the secondary spores may branch when still quite young, but as they continue to grow the protoplasm usually passes into one of the branches, leaving the others septate and empty. The end of the filament may become enlarged and rounded, while all the protoplasmic content of the hypha passes into it. The tip is then cut off by a cross wall. Sometimes, if the hyphae are large, they break up into a series of such cells (Pl. XL, fig. 5 g), and these have very much the appearance of chlamydospores. Old cultures consist almost entirely of these spores, and of empty, septate filaments (Pl. XL, figs. 5 f, g).

An examination of a culture derived from a single spore in vigorous growing condition shows that it consists of two kinds of hyphae: one type is  $5-8 \mu$  in thickness and  $80-150 \mu$  long, and filled with coarse granular material and oil globules; the other type is very slender and long,  $1-2 \times 200-400 \mu$ , with very fine granular contents. In the thick hyphae the protoplasm always moves towards the tip, the lower end becoming septate and empty. As the culture becomes older and the food exhausted, the protoplasmic content may mass to form a large two-celled structure (Pl. XL, fig. 5 e). The cells of either type of mycelium may become vacuolate and finally septate and assume a bamboo-like appearance. Single-celled spore-like bodies are often formed at the tips of the branches; these occur either singly or in chains. Both the one-celled spores and the two-celled structures, mentioned above, become surrounded by heavy walls, dark brown in color. They are filled with coarse material and oil globules. These structures have, then, all the morphological characteristics of chlamydospores. These spores measure from  $16$  to  $30 \mu$  in diameter. The one-celled spores germinate, when they are again supplied with nourishment, to form a branched or simple filament (Pl. XL, fig. 5 g). When two-celled bodies germinate, the wall separating the two cells is dissolved and the protoplasmic content passes into one of the two cells; this cell then forms a simple or branched hypha. The second type of mycelium is uniform in thickness, and the protoplasm usually extends throughout its length (Pl. XL, fig. 5 e); in the exceptions, series of little cells are formed, slightly larger in diameter than the filament. These structures have the appearance of oidia (Pl. XL, fig.

5 g). The fine mycelium may be considered as consisting of the younger hyphae of the fungus, because old cultures consist entirely of spore-like bodies and empty, septate, thicker hyphae.

### Tilletia Grown in Solutions

#### Carbohydrates

In order to determine the best combination of carbohydrates for mycelial production, *Tilletia Tritici* and *T. foetans* were grown in solutions of various concentrations of the following carbohydrates: dextrose, levulose, maltose, lactose, sucrose, commercial cane sugar, and malt extract. Of course, commercial cane sugar consists mostly of sucrose, but it was considered worth while to determine any possible difference between the most highly refined and the commercial article. Malt extract is a mixture, because it is a crude extraction of malted grains; although maltose constitutes the major portion, other carbohydrates, nitrogen compounds, and salts are present in varying amounts.

*T. Tritici* makes its initial growth during the first 10-12 days when placed in the following sugars: dextrose, maltose, levulose, lactose, sucrose, commercial cane sugar, and malt-extract solutions of about 5-percent concentration. Under these conditions it produces a small amount of mycelium; then growth ceases, and the mycelium begins to disintegrate. Nevertheless, there is a noticeable difference of growth in the various solutions. Their order, beginning with the best, is as follows: malt extract, dextrose, commercial cane sugar, maltose, levulose, sucrose, lactose. For mycelial growth, dextrose is better than maltose and both of these are much better than sucrose and levulose. Commercial cane sugar is not quite as good as dextrose, and malt extract is far superior to any of the sugars.

The concentration for optimum growth varies for each of the carbohydrates used, as is shown in table 4.

TABLE 4. Concentration of the Carbohydrates for Optimum Growth of *Tilletia Tritici*

Carbohydrate	Concentration
Dextrose.....	M/4
Levulose.....	M/2 to M/50
Maltose.....	M/4
Sucrose.....	M/8
Lactose.....	M/8
Commercial cane sugar.....	5 percent (about M/7)
Malt extract.....	5 to 10 percent

The temperature during this experiment was kept between 20 and 25° C. Under these conditions there is very poor growth in levulose, and the minimum concentration M/50, at which there is growth, is as good as any concentration up to M/2. This sugar seem to inhibit the production of mycelium. There was a good growth of mycelium in all concentrations of malt extract from 2 to 20 percent. It was impossible to decide which

concentration is best, and 5 percent was the concentration generally used. The minimum concentration at which growth will take place in any of these sugar solutions is between M/50 and M/100, while the maximum concentration for growth is between 2 and 3 M for maltose, and 4 and 5 M for dextrose. The minimum concentration for growth in malt extract was 0.03 percent, the maximum 80 percent.

#### *Addition of Salts to Carbohydrates*

Some salts when added to sugars promote the formation of mycelium, others inhibit it. Calcium nitrate added to malt extract or dextrose produced the best mycelial growth, and potassium nitrate was next best. Potassium dihydrogen phosphate and magnesium sulfate inhibit the growth of the mycelium. At very low concentrations magnesium sulfate is beneficial to mycelium-formation. The addition of calcium nitrate and potassium nitrate to a dextrose or malt-extract solution produces good mycelial development, but if potassium dihydrogen phosphate or magnesium sulfate is added a more or less inhibiting influence is exerted, depending upon the concentration.

Full solutions with any of the sugars used as the source of carbon produce good mycelium if the concentration of potassium dihydrogen phosphate and magnesium sulfate is kept very low, M/30 to M/40.

Malt extract gives very good results with or without salts. In fact, there is little difference in growth when salts are added.

The concentration of the salts was varied, and it was found that M/20 was the best concentration for all the salts except magnesium sulfate, for which M/40 was best. All the salts inhibit the development of the mycelium when used in concentrations greater than M/10.

Horse-dung decoction, whether used alone or with sugars, was found to be a very poor medium; in most cases there was no growth at all.

#### *Best Nutrient Solution for the Growth of the Mycelium of *Tilletia**

As a result of the above-described experiments, the best liquid medium for mycelial development was finally made with the following ingredients;

Calcium nitrate.....	0.4	gram
Potassium nitrate.....	0.2	gram
Potassium dihydrogen phosphate.....	0.2	gram
Magnesium sulfate.....	0.01	gram
Peptone.....	0.1	gram
Dextrose.....	2.0	grams
Malt extract.....	3.0	grams
Distilled water to 100 cc.		

As a final check on this medium, the concentration of all its constituents was also varied and tested. When the sugars are above 10 percent and salts above 5 percent, the development of the mycelium is inhibited. The most favorable concentration is about 5 percent of sugars and about 1 per-

cent of total salts, as shown above. But in no case, regardless of concentration and combination, was there any indication of the formation of chlamydospores or of spores of any other kind with these ingredients.

All cultures in the mycelial stage, when allowed to dry, turn black and the mycelium breaks up into little round cells with heavy walls. These spores will germinate to form mycelium when placed in the nutrient solution described above, or in a 3-percent solution of malt extract.

#### **Mycelial Development of *Tilletia Triticum* on Agar Media**

The development of the mycelium on agar media is better than in any of the nutrient solutions. The best agar media for the development of the mycelium are oatmeal agar, potato-sucrose agar, Leonian's agar, and malt-extract agar. Oatmeal agar is far superior to the others for mycelium-production. The largest production of mycelium takes place when the surface of the agar is moist and the atmosphere about the culture is saturated. Under such conditions the mycelium soon forms a thick white mat on the surface of the medium. As soon as the surface becomes dry, the development is checked. By "dry" is meant the condition when there is no free moisture at the surface of the agar, *i.e.*, when the water in the agar is in the adsorbed state.

#### **The Effect of Change of Conditions on Vigorously Growing Mycelium**

##### *Temperature*

Well developed cultures of *Tilletia Triticum* and *T. foetans* grown at room temperature (about 20° C.) on oatmeal agar, were placed at a temperature of 1 to 2° C. There was no change in the appearance of the fungus after three weeks. Other cultures were placed at a temperature of 25° C. At the end of a week the mycelium at the edge of the culture lost its snowy-white appearance and seemed to be dying. The center of the culture became brown and somewhat water-soaked. Examination showed that a temperature of 25° C. inhibits the development of the mycelium and causes it to disintegrate. At this temperature there is no sign of spore-formation. Some of the cultures were placed at 25° C. for 24 hours and were then placed at a lower temperature, 3 to 4° C., or left at room temperature. Changes in temperature caused no change in the kind of growth, but merely modified the rate of development.

##### *Carbon Dioxid*

The cultures of *Tilletia* were grown in an atmosphere containing various amounts of carbon dioxid. The lower percentages of carbon dioxid produced no visible change in the culture. In 75-percent carbon dioxid growth was inhibited, and in 100-percent carbon dioxid the mycelium began to disintegrate.



### Oxygen

The percentage of oxygen was changed by adding pure oxygen to the tube cultures or by exhausting the tube. The pressure was decreased in the tubes by steps of 100 mm. until a pressure of 30 mm. was obtained, the lowest that could be reached with the pump at hand. There was no change in the development of the mycelium. Increase in the percentage of oxygen appears to cause no appreciable change until approximately 100 percent is reached. After a few days at this concentration of oxygen, the mycelium begins to disintegrate. Oxygen is essential to the development of the fungus, but the amount needed is supplied as readily at low as at high oxygen pressure.

### CULTURAL STUDIES OF SPECIES OF USTILAGO

The genus *Ustilago* differs very greatly from the genus *Tilletia* in the physiological behavior of its species towards culture media. When spores of the species of *Ustilago* germinate, they form a promycelium that is 2- to 4-sepate and bears the sporidia at the septa. The sporidia reproduce by budding and form a large number of secondary spores. All the sporidia are uninucleate; all the secondary spores are very similar to the sporidia.

The following species of *Ustilago* were grown and studied: *U. Zeae*, *U. Hordei*, *U. Tritici*, and *U. Heufleri*.

### Growth in Sugar Solutions

Solutions of dextrose, levulose, lactose, maltose, sucrose, commercial cane sugar, and malt extract were used as culture media for these smuts. The smuts were grown in solutions of maltose with concentrations varying from M/1 to M/20. A concentration of M/7 was found to be best for the budding of sporidia and for the formation of secondary spores. The results are summarized in table 5.

TABLE 5. *Development of Species of Ustilago in Various Concentrations of Maltose*  
The minimum development is indicated by 1, the maximum by 4.

Maltose Concentration	<i>U. Zeae</i>	<i>U. Tritici</i>	<i>U. Hordei</i>	<i>U. Heufleri</i>
2 M.....	1	0	0	0
M/1.....	2	0	2	2
M/5.....	3	2	2	3
M/6.....	3	3	3	3
M/7.....	4	4	4	4
M/8.....	4	4	4	4
M/9.....	3	3	3	3
M/10.....	2	2	2	2
M/15.....	1	2	2	3
M/20.....	1	2	2	2

The table shows that for maltose the best concentration for the budding of the sporidia is between M/7 and M/8. One-seventh molecular has also



been found to be the optimum concentration for all the other sugars used. Sugars were sterilized at ten pounds' pressure for ten minutes.

An experiment was set up to determine the relative value of the different solutions and of a nutrient solution containing salts for the formation of secondary spores by budding. The concentration used for dextrose, levulose, maltose, and lactose was M/7; the concentration of commercial cane sugar and malt extract was 5 percent. A nutrient solution containing the following ingredients was also used:

Maltose.....	3	grams
Malt extract.....	3	grams
Potassium dihydrogen phosphate.....	1.25	grams
Magnesium sulfate.....	0.3	gram
Peptone.....	1.00	gram
Distilled water to 1,000 cc.		

The results are summarized in table 6.

TABLE 6. *Reaction of Species of Ustilago in Various Nutrient Solutions*  
The minimum development is indicated by 1, the maximum by 5.

	<i>U. Zeae</i>	<i>U. Tritici</i>	<i>U. Hordei</i>	<i>U. Henfleri</i>
Dextrose.....	1	2	1	2
Lactose.....	2	2	1	1
Levulose.....	2	2	3	0
Maltose.....	5	5	5	4
Malt extract.....	5	5	5	4
Cane sugar.....	3	2	1	1
Full solution 0.2 acid*.....	5	5	5	5

\* Potassium dihydrogen phosphate reacts as a buffer; the addition of small amounts of acid or alkali does not change the hydrogen-ion concentration to any appreciable extent.

The table shows that maltose is by far the best sugar for the development of the secondary spores of *Ustilago Zeae*. The secondary spores are large, and budding is very rapid. There is also some tendency to form resting spores; this takes place when the sporidia elongate, cease budding, and become swollen at one end but without the extensive formation of mycelium. There is very little budding of the secondary spores in levulose, lactose, or dextrose solutions. Dextrose is, furthermore, the lowest in nutritive value. Malt extract is better than maltose; the growth is very similar but more abundant. Cane sugar is not as good as maltose, but it is better than any of the other sugars.

*Ustilago Tritici* does not form sporidia in these solutions, but the promycelium breaks up into segments; therefore the comparative value of the sugar as a nutrient depends upon the number and size of the segments the fungus will form in a given solution. The table shows that maltose is much superior to the other sugars. Malt-extract solution is as good as maltose solution.

Table 6 shows that there is a slight growth of *Ustilago Hordei* in all the

sugars, but that in maltose it is much greater than in the others. In maltose the secondary spores bud rapidly and some mycelium is formed.

*Ustilago Heufleri* grows in all the sugar solutions except levulose, as shown by table 6. In dextrose and lactose solutions the secondary spores are very short and budding is infrequent. In maltose solution the budding is very rapid and many mycelial threads are formed. In cane sugar there is little or no budding of the secondary spores, and in a few days they turn black. Some of them conjugate, the protoplasmic content of one of the secondary spores passing into the other. There is an increase in size, and a heavy-walled spore is formed.

Maltose is by far the best sugar for the growth of the secondary spores of the smuts studied. Dextrose, levulose, and lactose are seen to have very little nutritive value. Although cane sugar is a little better than the three sugars just named, it is much inferior to maltose.

In the last row of table 6 are tabulated the reactions of the smuts to the salt-containing nutrient solution described just before the table. In this full nutrient solution many of the sporidia elongated, and the tips became swollen. The protoplasm of each sporidium passed into the swollen tip while the sporidium became vacuolate and finally empty and septate. The tip was cut off by a cross wall, and the empty part usually remained attached. In the full solution there was a difference in the behavior of the segments of *Ustilago Tritici*. They became much elongated and swollen at one end, and in some cases hyphae of considerable length were formed, with frequent fusion between the segments. Resting spores were formed in much the same manner as described for *U. Zeae*. The budding of the secondary spores of *Ustilago Heufleri* is inhibited somewhat by the full solution, and most of the secondary spores form germ tubes. A large amount of mycelium, made up of short septate hyphae, is produced. It forms a large mat which is white at first but soon turns black, and the hyphae break up into heavy-walled resting spores.

The addition of salts to maltose and to malt extract appears to influence favorably mycelium- and spore-formation and to inhibit budding. The salts were added to the sugars one at a time, and it was found that potassium nitrate and calcium nitrate in small amounts, *i.e.*, 0.01 to 0.02 percent, favored budding. Potassium dihydrogen phosphate and magnesium sulfate added to the sugars inhibited budding, and this inhibiting influence was strongest when they were added to maltose, malt extract, or levulose reactions. The addition of potassium dihydrogen phosphate and magnesium sulfate to maltose favors the formation of mycelium and causes the secondary spores of *Ustilago Heufleri* and *U. Hordei*, and the segments of *U. Tritici*, to conjugate and form resting spores. The mycelium may also break up into resting spores.

### Reaction of the Smuts to Acidity and Alkalinity

Most of the smuts grow best in a slightly acid medium. In order to determine the optimum reaction for the smuts, an experiment was set up, the results of which are given in table 7. An M/7 solution of maltose was made neutral to phenolphthalein, and the reaction of the different portions of it was changed by 0.1 percent of normal HCl or KOH.

TABLE 7. *Optimum Reaction for Development of the Species of Ustilago*  
Minimum growth indicated by 1, maximum by 4.

M/7 Maltose Reaction	<i>U. Zeae</i>	<i>U. Tritici</i>	<i>U. Hordei</i>	<i>U. Heufleri</i>
+0.6	0	0	0	0
+0.5	1	1	1	1
+0.4	2	2	2	2
+0.3	2	2	2	2
+0.2	3	3	3	3
+0.1	4	4	3	3
0.0	4	3	3	4
-0.1	2	2	2	4
-0.2	1	2	2	3
-0.3	0	1	0	2
-0.4	0	0	0	0

The maximum acidity at which budding still continues is about 0.5 percent, while 0.2 percent alkalinity inhibits budding of the sporidia and the secondary spores. The table shows that *U. Heufleri* grows better in an alkaline solution, but at the neutral point budding is inhibited and in the alkaline solution a large amount of mycelium is formed. The results of the experiment indicate that the optimum reaction for the budding of the secondary spores of the smuts studied is 0.1 percent acid.

### Alcoholic Fermentation

In order to determine whether or not the smuts ferment sugars, they were grown in the following solutions: dextrose, levulose, lactose, maltose, sucrose, commercial cane sugar, and malt extract. The sugars were sterilized at 10 pounds' pressure for 10 minutes, and experiments were set up as follows:

(1) Fifteen cc. of each solution was placed in a glass capsule of about 25 cc. volume, to which secondary spores of the smuts were transferred. One set was kept at room temperature and another at 29° C. At the end of 14 days the solutions were tested for ethyl alcohol.

(2) Small test tubes were nearly filled with the sugar solutions, and free access of the air was prevented by using very tight cotton stoppers and by dipping the stoppers in paraffin. One set was kept at room temperature, the other was placed in the incubator at 29° C. This latter experiment was set up because fermentation is often inhibited when the surface of the liquid is in contact with the air, or when the supply of oxygen is large.

After 14 days the solutions were tested for ethyl alcohol. The iodoform test was used to determine the presence or absence of alcohol in both cases. The test showed that none of the solutions had been fermented, since no ethyl alcohol was found.

### Solid Agar Media and Chlamydospore-formation

The smuts were grown on the following nutrient-agar and gelatin media: wheat, malted wheat, oatmeal, cornmeal, glycerin, Leonian's, malt extract, sucrose, potato-sucrose, sucrose-gelatin, and malt extract-gelatin. The smuts, both the species of *Tilletia* and of *Ustilago*, grow very well on any hard medium containing carbohydrates. The best medium for the vegetative growth of *Tilletia Tritici* and *T. foetans* is a heavy oatmeal agar. Malt-extract agar and Leonian's agar are very good also. The species of *Tilletia* form mycelium, and the sporidia of *Ustilago* species bud very profusely unless conditions of temperature, aëration, reaction, etc. of the medium are unfavorable. The growth of *Tilletia Tritici* and *T. foetans* on nutrient agars has already been dealt with; at this point the reaction of the species of *Ustilago* to solid media will be taken up.

The best medium for the production of sporidia by *Ustilago Zeae*, *U. Heufleri*, *U. Tritici*, and *U. Hordei* is one containing malt extract, peptone, calcium nitrate, and potassium nitrate. The promycelium of *U. Tritici* produces branches that break up into segments. In this respect it differs from the other species of *Ustilago*, in which sporidia are borne on the promycelium. The segments, however, bud in the same manner as other sporidia, provided the reaction of the medium is neutral or slightly acid.

The favoring temperature varies for the different species. The optimum temperature for growth of *U. Heufleri* is about 20° C., and its maximum is 36° to 37° C. *U. Zeae*, *U. Hordei*, and *U. Tritici* grow best at about 30° C. All four of these species gave very good results in culture on solid agar media.

If *Ustilago Zeae* is grown in a solution of maltose, to which potassium dihydrogen phosphate and magnesium sulfate have been added, many of the secondary spores conjugate. The same results can be obtained if the actively budding, secondary spores are placed on an alkaline glycerin agar. In about ten days a thin mat of mycelium is formed (Pl. XXXIX, fig. 3). The mycelium can be transferred to filter-paper pads (Leonian method) in capsules, in which there is a solution of maltose. The mycelium will continue to grow until a large amount is formed. When a good healthy growth of mycelium has been formed, the pad is transferred to sterile distilled water and washed several times in the course of 24 to 36 hours. The pads are then transferred to sterile capsules and placed in the incubator at 30° C. The mycelium breaks up into dark segments which have the appearance of chlamydospores. If left at room temperature, the chlamydospores are not formed.

*Ustilago Heufleri* grows very well on agar media containing carbohydrates, and prefers a neutral reaction. On a solid medium containing any of the sugars, or preferably maltose or malt extract, the sporidia multiply very rapidly by budding. If the cultures are allowed to grow for a long time, say two or three months, at a temperature of 28° to 30° C., there are formed large numbers of chlamydospores, the development of which can be brought about in a relatively short time if the conditions for vegetative growth are made unfavorable. When, also, the budding secondary spores are placed on an agar medium containing 1 to 2 percent glycerin with an alkaline reaction of 0.1 percent, large numbers of chlamydospores are formed. The secondary spores fuse to form a mycelium that grows within the agar, while very little if any is found at the surface of the culture; this mycelium finally breaks up into chains of chlamydospores. *These are formed in such large numbers that the agar appears black with them* (Pl. XXXIX, fig. 1). The spores are very characteristic; they are of the same size and shape as the chlamydospores formed in the plant, and they have the same structure, with an episporium and endosporium. They do not seem to germinate very readily, but those which have been observed in germination form a promycelium, which is 2- to 4-septate and bears sporidia laterally. *This smut can, therefore, complete its entire morphological life history in artificial culture.*

When the chlamydospores of *Ustilago Tritici*, obtained from its host, are germinated on the surface of a nutrient agar, a promycelium is formed; in 20 to 30 hours after germination many lateral branches can be observed on its sides. Both the promycelium and its branches are septate. The branches break up into segments that continue to reproduce by budding until a large number of segments result, so that the surface of the agar becomes covered by the segments. By transferring every four or five weeks, the smut can be grown for an indefinite period. As soon as the food supply is diminished, the segments form germ tubes which may grow to a length of 400 to 500  $\mu$ . The protoplasm remains at the tip of the tube, which is always somewhat swollen, and the rest of the filament becomes empty and septate. When the food is exhausted, large, round bodies, 6 to 9  $\mu$  in diameter, are formed at the tips of the branches and at the middle. When the culture is dry, each hypha has one or two chains of such spores, which are in every detail like the chlamydospores formed on the host plant. When the chlamydospores which were obtained in culture are placed under favorable conditions, they germinate by forming a promycelium that is septate, simple or branched, and breaks up into segments in the same manner as that derived from chlamydospores of the smut when growing on its host. *The chlamydospores of U. Tritici formed in culture must be considered true chlamydospores, since they behave similarly to those formed on its host plant.*

*Ustilago Hordei* is grown very easily. It germinates readily on maltose agar within 10 to 12 hours, and can be obtained in pure culture without any

trouble. It forms a promycelium which is usually 3- to 5-septate. Sporidia are produced in great numbers from the tip of the promycelium and from the septa, and the sporidia continue to multiply by budding. Soon the entire surface of the culture becomes covered by secondary spores. As the culture becomes older and the food becomes exhausted, the secondary spores form germ tubes; very often there is fusion between the secondary spores. The germ tubes may become very long, their protoplasm remains at the tip, and the rest of the filament becomes empty. When the food is exhausted, chlamydospores are formed. By growing the smut under the same conditions that were used in the culture of *Ustilago Zeae*, chlamydospores can be produced in two or three weeks. The chlamydospores germinate by forming promycelia, and sporidia at the septa. Sometimes the promycelium is short, with one or two septa or none at all (Pl. XL, fig. 11 c, d). *The chlamydospores formed in culture are similar to those obtained from the natural host.*

The smuts behave like other fungi; when conditions are favorable for vegetative growth, the sporidia and secondary spores bud continuously, but as soon as conditions are unfavorable, because of lack or withdrawal of food, spore-formation takes place.

#### GENERAL CONSIDERATIONS REGARDING INFECTION AND CULTURE

As indicated before, there have been very few attempts made to propagate the smuts continuously on artificial media. Brefeld (2, p. 13) cultivated the secondary spores of several smuts through successive generations for over a year; but they always continued to bud secondary spores, and formed scanty mycelium and no definite chlamydospores. However (p. 158), Brefeld, in his cultures of *Tilletia Tritici* in nutrient solution, noticed swollen bodies which resembled the young stages of chlamydospores. He never was able to show that these spores reached maturity. Stakman (20, pp. 18, 28) also obtained chlamydospore-like bodies in culture. He grew *Ustilago Tritici* and *U. Hordei* in a 5-percent solution of cane sugar, and when the food was nearly exhausted spore-like bodies were formed, which germinated when placed under favorable conditions. Kniep (12, p. 290) showed that *Urocystis Anemones* (Pers.) Wint. could be grown from chlamydospore to chlamydospore as a saprophyte on a malt-extract gelatin. The results of my own experiments show that probably all the smuts can be carried through their life history saprophytically. *Ustilago Heufleri*, *U. Hordei*, and *U. Tritici*, when grown on malt-extract agar, complete their life history in culture. The chlamydospores formed in culture are physiologically like the chlamydospores formed on the host, since, on germination, they form a normal promycelium. *Ustilago Zeae*, *Tilletia Tritici*, and *T. foetans* also form chlamydospores in culture, but these spores are physiologically unlike the chlamydospores formed on the host plant. They are like them in appearance, but the chlamydospores of *U. Zeae* do not form a



typical promycelium, while the chlamydospores of *Tilletia Tritici* and *T. foetans* do form a typical promycelium; however, the latter forms no sporidia, but secondary spores only. This difference may be due to the conditions under which the spores are formed, or the chlamydospores formed in culture may represent the types of spores produced by the old sexual form of conjugation of the sporidia. Lutman (14, p. 1222) says that "it is possible that at the present time the parasitic mycelium rarely or never starts from the conjugated conidia or promycelial cells even though they represent the old sexual form of conjugation." In any case it is quite possible that further experimentation might result in the discovery of conditions under which the life cycle of these three smuts as it occurs in nature could be exactly duplicated in culture.

The mycelium formed in culture on artificial hard media nearly always arises from the fusion of the sporidia or promycelial cells. Brefeld (2, p. 158) and Stakman (20, p. 42) have already described the formation of the germ tubes and mycelia of *Tilletia Tritici* and *T. foetans* in water and in nutrient solutions. Mycelium appeared, according to them, when the food supply was low or nearly exhausted. Kniep (12, p. 300) showed that *Ustilago Tritici* and *Urocystis Anemones* formed mycelium in a 1-percent solution of malt extract. At higher concentrations, little or no mycelium is formed. All the species of *Ustilago* studied by the writer reproduce by budding when the food supply is plentiful, but as soon as the food is nearly exhausted the mycelium begins to develop. With *U. Zeae* little or no mycelium is formed even when the solutions are very dilute, but if a transfer is made to a glycerin agar, mycelium forms immediately, since glycerin is much lower in food value than dextrose, maltose, or malt extract. Klebs (11, p. 106) has shown that for *Sporodinia grandis* Link 0.5 to 1 percent of cane sugar, maltose, or levulose is equivalent to 4 percent to 5 percent of glycerin. The transfer of the secondary spores of *U. Zeae* from a sugar solution to a glycerin agar is a change from a solution with a high and qualitatively different nutritive value to a medium with a low nutritive value of different quality. *Ustilago Tritici*, *U. Heufleri*, and *U. Hordei* will form some mycelium in a 3- to 5-percent solution of malt extract. When the culture is old, however, and the food is nearly exhausted, large amounts of mycelium are formed. *Tilletia Tritici* and *T. foetans* form mycelium in water, where the only food is that furnished by the spore. In nutrient solutions the germination of the chlamydospore is not normal, and no mycelium is formed. However, if the mycelium of *Tilletia* formed in water is transferred to a nutrient solution, as, for example, one of dextrose, large quantities of mycelium are formed. On nutrient agars, especially on oatmeal agar, *T. Tritici* and *T. foetans* form large mats of mycelium which continue to grow for several months if transfers are made occasionally. In this way the mycelium can be propagated for an indefinite period in a saprophytic condition.



Here we have a marked difference between the species of *Ustilago* and of *Tilletia*; the former produce budding sporidia when the food supply is large, and mycelium when the food supply is nearly exhausted; the latter, under the conditions of plenty of nutrition, form no budding sporidia but give rise to a well developed mycelium which bears secondary spores. There is also a difference in their growth in the host plant. The vegetative mycelium of the species of *Ustilago* is generally found at the growing points of the host, where food is concentrated and easily accessible. The fungus forms short strands of mycelium, and haustoria are wanting. The vegetative mycelium of species of *Tilletia* occurs in tissues less favorable for furnishing a concentrated food supply, *i.e.*, in the leaves and stems. The intercellular spaces are large, and the smut develops an extensive mycelium, with haustoria that penetrate the cells of the host. *Tilletia* forms mycelium more readily and more abundantly than *Ustilago*, both in the host and in culture.

When the mycelium is well nourished and nutriment is withdrawn, spore-formation begins. This is true for many fungi, and was considered a fundamental principle by Klebs (11, p. 101).

A topic related to the formation of mycelium and chlamydospores is the fusion of the sporidia. The sporidia of *Tilletia Tritici* and *T. foetans* fuse before they fall from the promycelium. In "*Ustilago carbo*" Tul., *Cintractia Montagnei* Tul., and *Ustilago Tritici* there is a fusion of the sporidia and of the segments of the promycelium; while in *Ustilago Zeae* there is no fusion of the segments of the promycelium nor of the sporidia when they are grown under similar conditions. The fusion of the sporidia may represent the old sexual form of conjugation which has ceased to play an essential rôle in the life cycle of the smuts, although it still occurs regularly when the promycelial cells or the sporidia are placed under conditions that favor it. Functionally, it may be that it has been replaced by the intracellular nuclear fusion in the chlamydospore.

From the results of recent studies of the smuts, it seems probable that there are different degrees in which the nuclear fusion occurs in the different species. In *Ustilago Tragopogonis-pratensis* Pers., according to Federly (5, pp. 1-23), the nuclei of the fused conidia travel toward each other and, as a rule, seem to fuse. In the promycelial cells of the species of *Ustilago* of the cereal grains, the nuclei move toward each other, come to lie in the same cell, and occasionally fuse. My observations show that this seems to be true for *Ustilago Heufleri*. In *Ustilago violacea* (Pers.) Fuckel, as described by Harper (7, pp. 482-487), the nucleus of each sporidium remains in its place, and all that occurs between the promycelial cells, sporidia, or secondary spores is a cytoplasmic fusion. Dangeard (3) has described a somewhat similar case in *Tilletia Tritici*, where the cytoplasmic fusion of the sporidia is never followed by a nuclear fusion. In addition to these cases in which fusions of various sorts occur, there are probably others in which no

conjugation of any kind, cytoplasmic or nuclear, takes place between the sporidia or the promycelial cells.

#### CONCERNING THE FURTHER LIFE HISTORY OF *USTILAGO HEUFLERI*

The mode of infection by this smut, and its reactions under various cultural conditions, have been given, for comparative purposes, in the preceding pages. Here will be taken up the germination of the spores and the nuclear history in so far as the latter could be determined.

##### Dormancy of the Chlamydospores

The fresh spores of *Ustilago Heufleri* which were collected in May, 1922, did not germinate, and those kept over winter in the laboratory or out of doors did little better; a very few spores were observed to germinate. Fresh spores were gathered in May and June of the succeeding year, but they also failed to germinate. The following procedure, therefore, was tried, and successful germinations were obtained.

Some of the smutted leaves were allowed to dry for three or four weeks and then were placed in a moist chamber for a few days. The moist pustules were cut out and placed in test tubes. Enough distilled water was added to cover the material, and the tubes were placed in an ice bath. When the water was frozen, the tubes were removed from the ice bath and placed in the refrigerator. After two days they were removed, and the water was drained off. The spore pustules were placed in bottles, and the bottles were attached to an oxygen generator. The oxygen was allowed to pass in until all the air was replaced by oxygen. The bottles were sealed, and the spores were left in the oxygen for from 1 to 24 hours. At intervals of two hours, spores were removed from successive bottles and placed in hanging drop cultures. The spores which had been oxygenated for 10 hours germinated in 12 to 16 hours. The spores were germinated in water, dextrose solution, and on malt-extract agar. They germinated best at temperatures from 15° to 20° C., there being no germination at 30° C. or higher.

##### Germination in Water

The promycelium breaks through the spore wall without rupturing it. When full grown, the promycelium is 2- to 4-septate, and measures 70–80  $\times$  4–5  $\mu$ . The sporidia are formed at the septa, but they were not observed to form secondary spores by budding. The sporidia, while still attached, or often after detachment, send out germ tubes, protoplasmic at first, but soon becoming vacuolated or entirely empty and segmented in the basal portion. After 24 hours some of the sporidia have already germinated. Most of them, however, require a much longer time. The filament at first is narrow, but as growth progresses these infection threads assume practically the same character as those sent out by the promycelial segments. The

sporidia themselves become empty after the germ tubes are well established. Very little change, if any, was observed after 4 to 5 days.

#### Germination in Nutrient Solution

The germination in nutrient solution is very similar to that in water except that the promycelium is somewhat larger, and the sporidia form many secondary spores by budding. They continue to bud as long as the nutriment lasts, forming large numbers of secondary spores. Upon germination the secondary spores give rise to slender germ tubes, resembling quite closely those formed in water.

#### Germination on Malt-extract Agar

At 20° C. the chlamydospores germinate in 20 to 24 hours. The promycelium is 2- to 3-septate, 70-80  $\times$  4-5  $\mu$  in size, usually simple but at times with two or three branches. The sporidia immediately reproduce by budding, forming a large colony which appears at the surface of the agar. The sporidia and secondary spores continue to bud while the nutriment lasts, and the colony continues to enlarge. At first the colony is white and moist, but as it becomes older it turns brown to dark brown. After 20 to 30 days, many of the secondary spores germinate, forming germ tubes. At first the germ tube is long and filled with fine, granular protoplasm; but soon the tip becomes swollen, the protoplasm flowing into it, while the basal portion becomes septate and empty. When the nourishment is exhausted, the tip is divided into segments by definite cross walls. The segments, which are similar in every respect to chlamydospores, round up, form a heavy wall, and become dark brown. They measure from 14 to 20  $\mu$  (Pl. XLI, figs. 13 c, d), and under favorable conditions germinate to form a promycelium. Sporidia are formed at the septa (Pl. XLI, fig. 13 e). Many of the secondary spores may never form germ tubes, but when the food is exhausted they surround themselves with a heavy wall and become dark brown in color, while under favorable conditions they reproduce by budding. Very often the secondary spores fuse in pairs (Pl. XLI, fig. 13 b).

#### THE CYTOLOGY OF USTILAGO HEUFLERI

The cytology of the smuts is better known than their behavior in culture. There is a fairly complete account of the nuclear phenomena of the following smuts: *Ustilago Zeae*, *U. Tritic*, *U. levis* (Kell. & Sw.) Magn., *U. nuda* (Jens.) Kell. & Sw. *U. Hordei*, *U. Tragopogonis-pratensis*, *U. violacea*, *U. Avenae*, *Cintractia Montagnei*, *Doassansia Sagittariae*, *D. Alismatis*, and *Urocystis Anemones*. Partial accounts are available for *U. longissima* (Sow.) Tul., *Doassansia deformans* Setch., *Doassansia Nymphaeae*, *Entyloma Nymphaeae* (D. D. Cunn.) Setch., and *Tilletia Tritic*. Rawitscher (18) has given a coherent account of the nuclear behavior of *Tilletia Tritic*, but he

was not able to determine the nuclear condition of the secondary spores when these occur. Dangeard (3), however, states that the secondary spores are always binucleate, but this has not been verified.

Since the chlamydospores of *Ustilago Heufleri* were obtained in culture, it became a matter of interest to determine whether or not the nuclear conditions of the spores formed in culture and of those formed in the host plant were the same. The chlamydospores from cultures were germinated on malt-extract agar, in water, and in a 5-percent solution of dextrose. The germinated spores were placed on a clean slide which had been covered with a thin film of Szombdathy's fixative. A drop of strong Flemming's solution was placed on the spores and allowed to remain for 20 to 30 minutes. The killing fluid hardens the fixative, so the spores are held fast to the slide. The preparations were washed in distilled water for 1 to 2 hours, then bleached in hydrogen peroxid, and stained in Heidenhain's haematoxylin. A few of the preparations were stained in Flemming's triple stain.

The mature chlamydospore contains a single nucleus (Pl. XLI, fig. 12 *d*). When the spore germinates it forms a 2- to 4-septate promycelium. The nucleus passes into the promycelium and two divisions occur to form four nuclei, which become separated by cross walls. Each cell of the promycelium contains one nucleus (Pl. XL, fig. 9 *a*). The sporidia are formed at the septa and at the tip of the promycelium. All the secondary spores formed by budding are uninucleate. In old cultures the secondary spores fuse in pairs to form binucleate resting spores (Pl. XLI, fig. 13 *d*), and when these are placed on malt-extract agar they germinate to form a promycelium that bears sporidia. In many cases a binucleate or multinucleate mycelium is formed. When the food in the medium is exhausted the mycelium breaks up into segments, which usually fuse in pairs to form large resting spores (Pl. XLI, fig. 13 *c*). In some cases more than two segments fuse, so that the resting spores are multinucleate. At times the nuclei in each spore are very close together, but it is very difficult to determine whether or not they fuse (Pl. XLI, fig. 13 *d*). In every case of fusion the entire contents of one cell passes into the other, and there is a cytoplasmic growth in size (Pl. XLI, fig. 13 *bb*). When the chlamydospores which were formed in culture were placed on a malt-extract agar, they germinated to form a 2- to 3-septate promycelium, and sporidia were formed at the septa and at the tip of the promycelium. Many of the spores, however, formed sporidia directly.

It is rather difficult to obtain early stages in the development of the fungus in the host. When the light spots which indicate points of infection appear on the leaves, the disease is well advanced and the mycelium found there is fully developed and breaking up into spores.

The youngest pustules that could be found were cut out and placed in strong Flemming's or medium chrom-acetic killing fluid. The bottles were attached to an exhaust pump and all the air was drawn out of the pieces of leaves, allowing the killing fluid to penetrate quickly to all parts of the

material. The material was left in the fixing fluid for 20 to 24 hours and then washed in running water for 24 hours. It was passed through eighteen grades of alcohol, being left in each of the lower concentrations for about 1/2 hour, in 70, 85, and 95 percent alcohol for 12 hours each, and in absolute alcohol for 12 hours. Then cedar oil was placed in the bottle by means of a pipette, so as to make a layer at the bottom of the absolute alcohol. When the material had sunk into the cedar oil, the alcohol was decanted and fresh cedar oil was added. After 10 to 12 hours, the material was placed in xylol and imbedded in the usual manner. The material was then sectioned, stained with Heidenhain's haematoxylin, and mounted in balsam.

The mycelium found in the plant is always intercellular. It does not spread very far from the point of infection, and is never found a centimeter beyond the pustule. In the very youngest pustules a mass of mycelium is found in the intercellular spaces just below the epidermis, and the mycelium extends in all directions from the point of infection. The mycelium usually extends to the opposite epidermis, and a little farther in the other directions. The greatest growth is lengthwise of the leaf, following the tracheae, which accounts for the formation of the elongated pustules. The largest amount of mycelium is formed at the point of infection, the hyphae growing between the cells, pushing them apart, and distorting them. Finally the host cells disintegrate, and the space becomes filled with mycelium. The hyphae at the center of the pustule break up, into segments first, and segmentation proceeds towards the periphery until all the hyphae have broken up into spores. In a mature pustule no mycelium is left, and only a few hyphae are found between the cells adjoining the pustule. The entire process, from the first appearance of the pustule to the maturing of chlamydospores, takes place in a week or ten days.

The mycelium found in the plant is usually multinucleate, and the nuclei are nearly always associated in loose pairs (Pl. XLI, fig. 12 *a*). In the early stages the mycelium is 3 to 5  $\mu$  in diameter and several hundred  $\mu$  long, branched, and filled with a fine, granular protoplasm. Just before the hyphae break up into segments they enlarge greatly. Most of the segments formed contain two nuclei each. There are, however, some segments cut off that appear to have no nuclei (Pl. XLI, fig. 12 *b*). These empty segments are often found attached to the mature spores. The segments are cut off from the ends of short lateral branches or from the end of the main branch, and the process proceeds backwards until all the mycelium is used. Just before segmentation the tip of the branch enlarges, two nuclei pass in, and are cut off by a cross wall. The segment continues to enlarge, and begins to round up before it becomes free (Pl. XLI, fig. 12 *b*). At this time the wall thickens and the entire surface becomes gelatinized, so that it is not possible to determine the behavior of the nuclei at this point in the life history. It is very probable that the nuclei fuse at this time, because a little later nearly all the segments are uninucleate. The thick episporium and the



endosporium are now completely formed. In a very few cases, at this stage, spores have been found with two nuclei (Pl. XLI, fig. 12 *e*). In some of the spores the nuclei are very close together, and seem to be in the act of fusing. It is very likely that the mature chlamydospore contains a fusion nucleus (Pl. XLI, fig. 12 *d*).

When the chlamydospore obtained from the host plant germinates, the fusion nucleus passes into the promycelium and divides to form four daughter nuclei (Pl. XL, fig. 9 *a*). One of these nuclei is present in each cell of the promycelium. The sporidia and all the secondary spores formed by budding are uninucleate (Pl. XL, fig. 9 *b*). To sum up, the mycelium in the host is multinucleate and breaks up into binucleate segments: the nuclei apparently fuse before the chlamydospores are mature, the mature chlamydospores thus being uninucleate.

I have previously discussed the fusion of the sporidia of the smuts, and have noted that there may be different degrees of fusion in different species. The work with *Ustilago Heufleri* shows that there is a nuclear and cytoplasmic fusion of the sporidia and of the secondary spores. Each spore resulting from the fusion of sporidia contains two nuclei at first; later, when the spore is mature, one nucleus is present. This same condition exists when the chlamydospores are formed from the hyphae in culture; the segments are at first binucleate, and the nuclei fuse before the spore is mature.

The nuclear condition of the germ tube that enters the host was not determined for *Ustilago Heufleri*. In some smuts, according to Rawitscher (17, p. 687), the mycelium that enters the host is uninucleate, as in *U. Zeae*, and fusion of the short hyphae in the plant gives rise to the binucleate condition. In other smuts, like *U. Hordei*—“*U. carbo*” (17, pp. 691–697), the mycelium that enters the host is binucleate. This latter is probably true in the case of *U. Heufleri*, because at no time has a uninucleate mycelium been found in the tissues of the host, and no fusion of the mycelium at or before the time of spore-formation could be recognized. However, the nucleus in the mycelium may divide without a division of the cell; in this way the cells might become multinucleate without necessarily becoming sporophytic. Lutman (14, p. 1220), says that:

Nuclear division may continue until the cell contains twenty or thirty sister nuclei. The sporophytic generation cannot be assumed to begin until the binucleated condition has been definitely established.

Rawitscher, in all his work, studied the mycelium of the smut when it was uninucleate or distinctly binucleate, as at the time of spore-formation. Lutman (p. 1220), however, in speaking of the nuclear condition of the smuts, says:

In general it may be said at the present time as to the nuclear condition of the smut mycelium that in the genus *Ustilago* the cells are multinucleated up to the time of spore formation; in some of the *Tilletia* group the cells are certainly binucleated while in others they are probably so, at least to the extent that many binucleated cells are present.

The nuclear condition that he found in species of *Ustilago* is the one that exists in the mycelium of *U. Heufleri*. The cells of the mycelium are multinucleate, but with the nuclei in loose pairs. At the time of spore-formation the mycelium breaks up into binucleate segments. These segments become chlamydospores, and the nuclei fuse before the spores are mature. In all the species of smuts studied, the chlamydospores arise from binucleate segments of the mycelium, and there must be an intracellular fusion of the nuclei of the young chlamydospore; for the mature chlamydospore contains a single nucleus.

#### GENERAL SUMMARY

1. The outstanding result of the writer's work on the smuts is the evidence presented that *Ustilago Hordei*, *U. Tritic*i, and *U. Heufleri* can complete their morphological life history saprophytically in artificial culture media. This amplification of the recent work of Kniep with *Urocystis Anemones* leads to the conclusion that careful enough adjustment of the environmental requirements may make it possible to bring about the same results in all the smuts, thus showing that no portion of their life cycle is obligatively parasitic.

2. The development of mycelium, comparable with that of other fungi in culture, depends only, as shown, upon the presence of an appropriate medium and of otherwise optimum conditions. Mycelium is formed by *Ustilago Zeae*, for example, when the secondary spores are placed on glycerin agar; when this mycelium is afterward transferred to a 5-percent maltose solution under special conditions, large amounts of mycelium are formed.

3. The mycelium of *Tilletia Tritic*i and of *Tilletia foetans* develops best on a heavy oatmeal agar; malt extract in solution is the best liquid medium, and dextrose forms the best sugar solution for this purpose. The addition of calcium nitrate and potassium nitrate to the solution favors the formation of mycelium, while magnesium sulfate and potassium dihydrogen phosphate inhibit its formation.

4. The formation of sporidia and secondary spores, in the species of *Ustilago* studied, is most abundant on agars containing malt extract, peptone, calcium nitrate, and potassium nitrate; here the two salts inhibit rather than promote mycelial development. Of the sugars, maltose in solution is by far the best in promoting a rapid budding of secondary spores; dextrose, levulose, and lactose have very little effect.

5. Potassium hydrogen phosphate and magnesium sulfate, while acting negatively on *Tilletia*, promote the formation of mycelium and cause the secondary spores of *Ustilago Hordei* and *Ustilago Heufleri* to conjugate and form resting spores, when added to a maltose solution.

6. Resting spores of *Tilletia Tritic*i do not form in nutrient solution, but appear when the food supply becomes exhausted. These spores have all the appearance of chlamydospores except that they do not form sporidia on germination, but mycelium and secondary spores only.



7. The smuts studied develop best on a slightly acid medium.  
8. Sugars are not fermented by the four species of *Ustilago* studied.  
9. The full life history of *Ustilago Heufleri* is here presented for the first time. Its mycelium in the host plant is multinucleate until just preceding spore-formation, when it breaks up into binucleate hyphae that segment and round up into binucleate chlamydospores. The two nuclei in each spore fuse before the chlamydospore is mature. The chlamydospore germinates to produce a 2-4-septate promycelium, which in turn forms sporidia at the septa.

10. The sporidia and secondary spores of *Ustilago Heufleri* formed in culture are uninucleate. In old cultures they frequently fuse in pairs, the nucleus and cytoplasm of one secondary spore passing into the other; when the food supply becomes exhausted, the mycelium present breaks up into segments which may also fuse in pairs and form chlamydospores. The secondary spores may produce hyphae directly, and these may break up into binucleate segments to form chlamydospores. The chlamydospores from both sources can germinate to form promycelia.

11. The fresh chlamydospores of *Ustilago Heufleri* gathered in spring did not germinate; when, however, they were frozen for a few days and then treated with oxygen for 24 hours, they germinated readily in water, in dextrose solution, and on malt-extract agar.

12. The infection of *Erythronium americanum* by *Ustilago Heufleri* takes place when the leaves of the host are just appearing through the soil. The smut can not infect the leaves that are noticeably above the ground.

13. Infection of wheat by *Tilletia Tritici* occurs from the time of germination until a few days after the germination of the wheat, as reported by others. The germ tube from the fused sporidia enters between the cells of the sheath leaf; it is very seldom that the germ tube is intracellular.

14. The mycelium and secondary spores of *Tilletia Tritici* are not able to infect the host at least at the time and place where infection occurs from the usual infection tube.

15. The mycelium and resting spores of *Ustilago Zeae*, formed in culture, are not able to infect the host.

These investigations were carried on in the cryptogamic laboratory of the University of Michigan under the direction of Prof. C. H. Kauffman, to whom the author wishes to express his gratitude for his interest in the work and his helpful constructive criticisms. I also wish to thank Prof. J. H. Hotson of the University of Washington for materials and helpful criticism.

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For a more complete bibliography of the literature of the smuts the student is referred to the work of Lutman (14).

## DESCRIPTION OF PLATES

### PLATE XXXIX

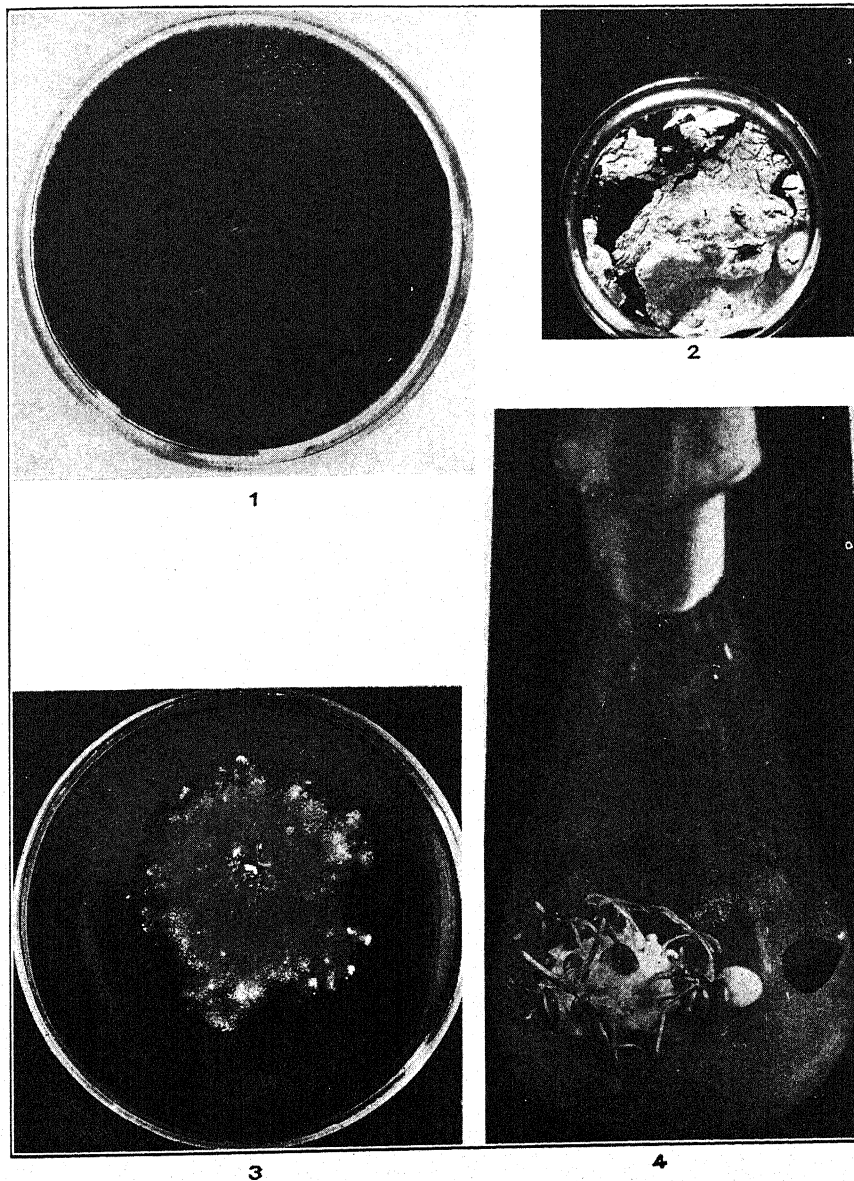
Photographs reduced  $\frac{1}{2}$

- FIG. 1. Culture of *Ustilago Heufleri* showing the formation of chlamydospores.  
 FIG. 2. The vegetative mycelium of *Tilletia Tritici* developed on sterile wheat kernels.  
 FIG. 3. The mycelium of *Ustilago Zeae* formed on glycerin agar.  
 FIG. 4. The vegetative mycelium of *Tilletia Tritici* formed on nutrient agar. Sterile wheat seedlings growing on the culture.

### PLATE XL

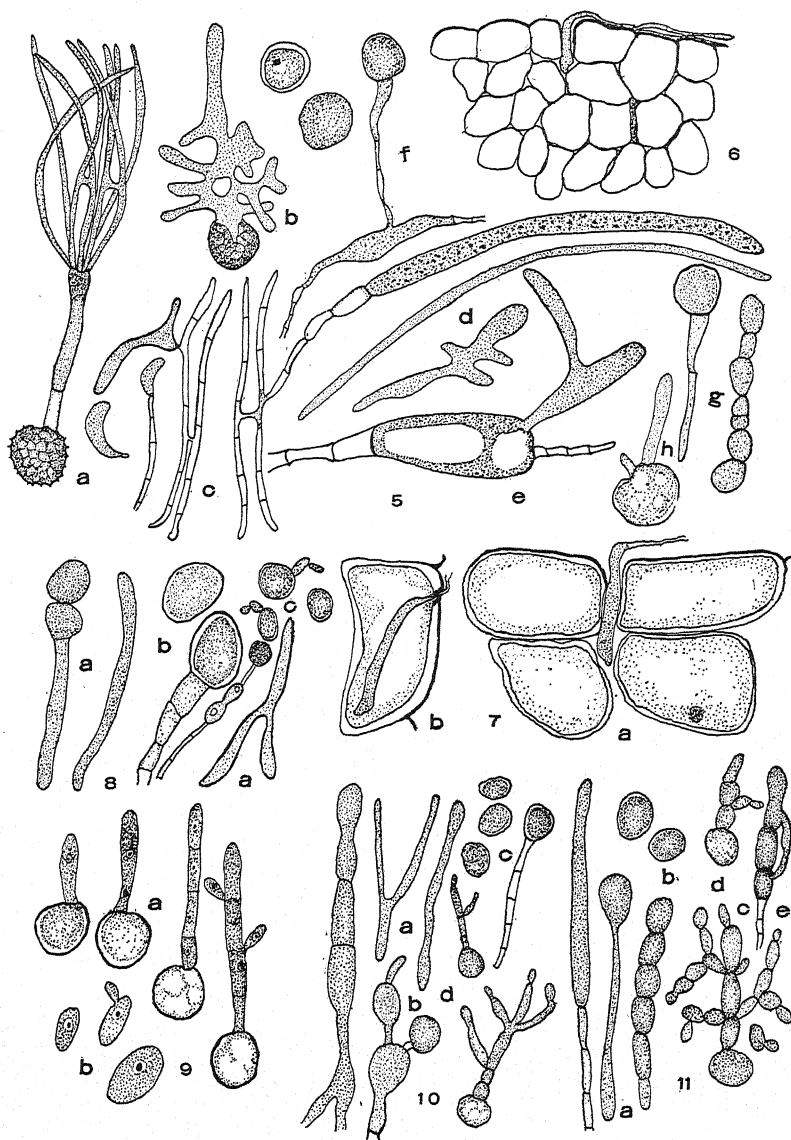
Bausch and Lomb 15 X ocular and 3-mm. objective were used (X 700), except for figure 6, which was drawn with an 8-mm. objective (X 350).

FIG. 5. *a*, Chlamydospore of *Tilletia Tritici* germinated on malt-extract agar. *b*, Abnormal germination of chlamydospores. *c*, Fused sporidia and secondary spores. *d*, Large and small hyphae formed in culture. *e*, A large resting spore germinating. *g*, Oidia obtained from a culture on agar. *f*, *h*, Chlamydospores, formed in culture, germinating.



3 4  
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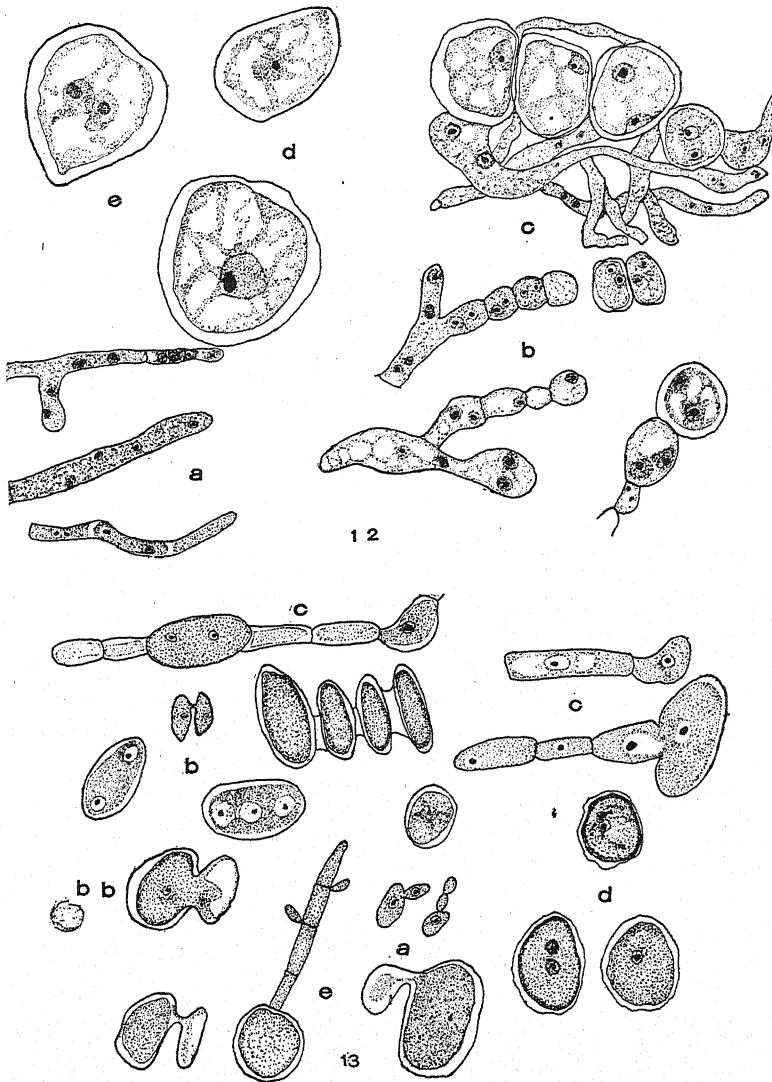




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FIG. 6. A germ tube of *Tilletia Tritici* entering between the cells of the sheath of a wheat seedling.

FIG. 7. *a*, Same as a portion of figure 6, enlarged. *b*, The germ tube of *Tilletia Tritici* entering a cell of the sheath.

FIG. 8. *a*, Mycelium of *Ustilago Zeae* formed on glycerin agar. *b*, Large resting spores formed at the tip of a hypha. *c*, Small resting spores of the same size as the chlamydospores formed on the host; two are germinating.

FIG. 9. *a*, Stages in the germination of the chlamydospores of *Ustilago Heufleri*. *b*, Uninucleate sporidia and secondary spores.

FIG. 10. *a*, Mycelium of *Ustilago Tritici* formed in culture. *b*, The swollen end of a germ tube. *c*, Chlamydospores formed in culture. *d*, Germination of a chlamydospore formed in culture.

FIG. 11. *a*, Mycelium of *Ustilago Hordei* formed in culture. *b*, Chlamydospores and their formation in culture. *c*, Chlamydospores derived from the host germinating on nutrient agar. *d*, Germination of a chlamydospore formed in culture. *e*, Fusion of two cells in culture.

#### PLATE XLI

Figure 12 was drawn with a Bausch and Lomb 1.9-mm. oil-immersion objective and a 15 × ocular (× 1,200). Figure 13 was drawn with a 3-mm. objective and a 15 × ocular (× 700).

FIG. 12. *a*, Multinucleate mycelium of *Ustilago Heufleri* formed in a leaf of *Erythronium americanum*. *b*, The mycelium breaking up into binucleate segments. *c*, The segments rounding up to form chlamydospores. *d*, Mature uninucleate chlamydospores. *e*, Germination of a chlamydospore formed in culture.

FIG. 13. *a*, Uninucleate budding secondary spores of *Ustilago Heufleri*. *b* and *bb*, Stages in the conjugation of the secondary spores (from a culture). *c*, Septate mycelium formed on agar media, and the fusion of two of the cells of such a mycelium. *d*, Younger and older chlamydospores (from culture). *e*, Germination of a chlamydospore formed in culture.

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